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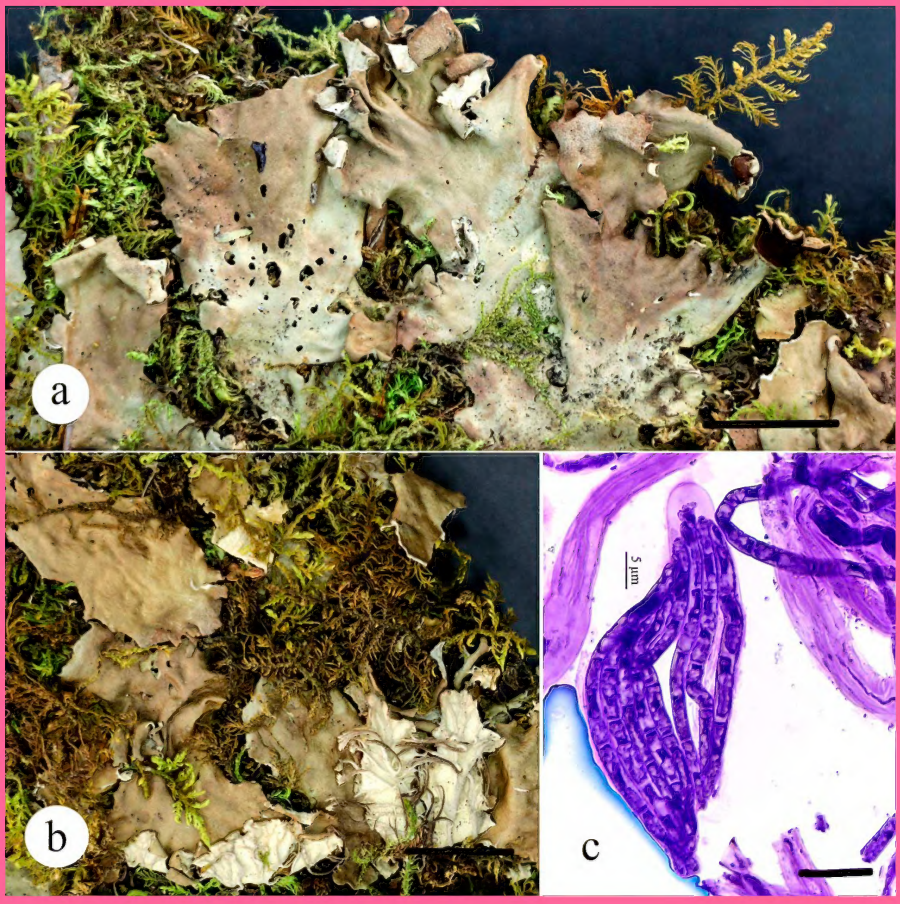

Peltigera neodegenii sp. nov.

(Han, Yu, Zhang, Guo— FIG. 3, p. 329)

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MYCOTAXON

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Errata from previous volumes

Volume 111

p. 364, line 29	for: (TENN63392)	read: (TENN63485)
p. 367, line 10	for (TENN63390)	read: (TENN63483)
p. 367, line 11	for: (TENN63393)	read: (TENN63476)
p. 367, line 12	for: (TENN63391)	read: (TENN63486)
p. 367, line 13	for: (TENN63385)	read: (TENN63480)
	for: (TENN63386)	read: (TENN63479)
p. 367, line 14	for: (TENN63387)	read: (TENN63482)
p. 367, line 15	for: (TENN63388)	read: (TENN63481)
	for: (TENN63389)	read: (TENN63484)

Volume 130(4)

p.1032, line 1	for: HMAS 264195	read: HMAS 266592

Volume 133(1)

p.113, (Abstract, line 2)	for: *Caloplaca zeorina*,*Verrucaria eminens* read: *Caloplaca zeorina*, and *Verrucaria eminens*	
p.113, (Intro, line 2)	for: Qinghai province	read: Qinghai Province
p.116, (Caption, line 6)	delete: A dash indicates branches with posterior probabilities <95%.	
p.128, line 36	for: 30'00"E	read: 30′00″E

Nomenclatural novelties and typifications proposed in Mycotaxon 133(2)

Alternaria allii-tuberosi (X.G. Zhang & T.Y. Zhang) Gannibal & D.P. Lawr.
[MB 822967], p. 295

Alternaria castaneae (X.G. Zhang & T.Y. Zhang) Gannibal & D.P. Lawr.
[MB 822968], p. 295

Alternaria gpagarwalii (Nagaraju, Kunwar, Manohar. & D.K. Agarwal) Gannibal & D.P. Lawr.
[MB 822966], p. 295

Alternaria manihoticola (J.M. Yen) Gannibal & D.P. Lawr.
[MB 822970], p. 295

Alternaria microspora (Moub. & Abdel-Hafez) Gannibal & D.P. Lawr.
[MB 822971], p. 295

Alternaria oblongo-obovoidea (X.G. Zhang & T.Y. Zhang) Gannibal & D.P. Lawr.
[MB 822972], p. 295

Alternaria populicola Gannibal & D.P. Lawr.
[MB 822973], p. 296
≡ *Ulocladium populi* E.G. Simmons, G. Newc. & A. Shipunov 2009
non *Alternaria populi* T.Y. Zhang 2003

Alternaria preussii Gannibal & D.P. Lawr.
[MB 822974], p. 296
≡ *Ulocladium dauci* E.G. Simmons 1999
non *Alternaria dauci* (J.G. Kühn.) J.W. Groves & Skolko 1944

Alternaria pseudobotrytis Gannibal & D.P. Lawr.
[MB 822969], p. 296
≡ *Ulocladium leve* H.M Liu & T.Y. Zhang 2008
non *Alternaria levis* Gambogi ex E.G. Simmons 2007

Alternaria sorghi (X.G. Zhang & T.Y. Zhang) Gannibal & D.P. Lawr.
[MB 822975], p. 296

Alternaria sylvestris Gannibal & D.P. Lawr.
[MB 822976], p. 296
≡ *Ulocladium lignicola* Nagaraju, Kunwar, Monohar. & D.K. Agarwal 2009
non *Alternaria lignicola* (Corda) Fr. 1849

Alternaria zantedeschiae (X.G. Zhang & T.Y. Zhang) Gannibal & D.P. Lawr.
[MB 822977], p. 296

Ascotricha funiculosa (Guarro & Calvo) D.W. Li & G.H Zhao
[MB 824983], p. 227

Ascotricha microspora D.W. Li & G.H Zhao
[MB 822539], p. 224

Ascotricha rugispora (Okane, Nakagiri & Tad. Ito) D.W. Li & G.H Zhao
[MB 822541], p. 226
Craspedodidymum guatemalense Figueroa, Bran, O. Morales & R.F. Castañeda
[IF 554791], p. 316
Endophragmiella terricola J.J. Xu & T.Y. Zhang
[MB 823960], p. 301
Gliomastix verrucipes T.Y. Zhang, Y.M. Wu & J.J. Xu
[MB 823962], p. 303
Lasiodiplodia cinnamomi C.M. Tian & Ning Jiang
[MB 822574], p. 255
Leucoagaricus pabbiensis Usman & Khalid
[MB 824295], p. 358
Neopestalotiopsis rosicola C.M. Tian & Ning Jiang
[MB 820879], p. 277
Peltigera neodegenii L.F. Han, S.Y. Guo & Xiao M. Xu
[MB 821289], p. 328
Phylacia cylindrica L.T. Lacerda, J.L. Bezerra & Jad. Pereira
[MB 805788], p. 244
Radulidium guttiforme T.Y. Zhang, Y.M. Wu & J.H. Kong
[MB 823963], p. 304
Rhexoampullifera marquesii L.B. Conc. & Gusmão
[MB 824255], p. 350
Tolypocladium dujiaolongae Y.P. Cao & C.R. Li
[MB 821894], p. 7

Reviewers — volume one hundred thirty-three (2)

The Editors express their appreciation to the following individuals who have, prior to acceptance for publication, reviewed one or more of the papers prepared for this quarter.

Rafael F. Castañeda-Ruiz
Elsie Cruywagen
Cvetomir M. Denchev
Maria Martha Dios
Francisco C.O. Freire
Gabriela Heredia Abarca
Adriana Hladki
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Yue-Li Zhang

publication date for volume one hundred thirty-three (1)

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was issued on April 25, 2018

From the Editor-in-Chief

Yes! Mycotaxon 133(2) is three months late. And very slim! —Illustrious founders Korf & Hennebert never intended for a Mycotaxon journal to arrive so tardily nor with so few pages. Both lateness and size were caused by unhappy circumstances: a family illness and death moved journal preparations off schedule for four months, and 2018 final manuscript submissions are fewer than since before 2004. Nonetheless, rest assured—we will bring the journal back on schedule by continuing to publish papers in the order received within each three-month period. We still plan to publish four issues during 2018.

No more required page charges! —Fortunately, Korf & Hennebert would heartily approve of our lifting the page charges we were forced to adopt in 2011. Mycotaxon is delighted to announce that we now return to a subscription payment model. As of September 15, there will be no page charges required from an author for publishing a paper in the journal. Now the only payments due Mycotaxon are for annual private and institutional subscriptions, optional open access, correction of major author errors in PDF proofs, and posting a mycobiota on our website (www.mycotaxon.com).

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The author error correction fee ($40 per error) is an obvious editorial ploy to convince our authors to strive for perfection: if all coauthors, two expert peer reviewers, and two editors revise a paper, there really should be no mistakes to 'discover' the first time in a PDF proof. Your unpaid editors crave your assistance!

Mycotaxon 133(2) contains 19 papers presented by 59 authors (representing 15 countries) and revised by 35 expert reviewers.

Within its pages are 12 species new to science representing *Ascotricha* from Cayman Islands; *Craspedodidymum* from Guatemala; *Endophragmiella, Gliomastix, Lasiodiplodia, Neopestalotiopsis, Peltigera, Radulidium,* and *Tolypocladium* from China; *Leucoagaricus* from Pakistan; and *Phylacia* and *Rhexoampullifera* from Brazil.

In addition to 14 new combinations in *Alternaria* and *Ascotricha,* we also offer a revision of three smut fungi from Japan, two papers on rusts from China covering intracellular spermogonia in one *Uromyces* and a *Puccinia* with albino teliospores,

a second world record of the myxomycete *Arcyriatella congregata* in Mexico, exploration of the *Heterobasidion insulare* complex in Pakistan, and two excellent papers devoted to the sectionalizing of *Alternaria* (including the disposition of species formerly assigned to *Ulocladium*). We also summarize an impressive checklist of asexual fungi from Costa Rica new to the Mycotaxon mycobiota webpage.

Warm regards,

Lorelei Norvell (*Editor-in-Chief*)
21 September 2018

2018 Mycotaxon submission procedure

Prospective Mycotaxon authors should download the Mycotaxon 2018 guide, review & submission forms, and Mycotaxon sample manuscript by clicking the 'file download page' link on our **Instructions to Authors** page before preparing their manuscript. This page summarizes our '4-step' submission process.

1—Peer review: Authors first contact two (for journal papers) or three (for annotated species 'weblists') peer reviewers before sending them formatted text & illustration files and the appropriate 2018 Mycotaxon journal or weblist reviewer comment form. Experts **must** return revised manuscripts & comments to **both** the *Editor-in-Chief* <editor@mycotaxon.com> and authors. The authors correct their files before submitting their manuscript to the *Nomenclature Editor*.

2—Nomenclatural review: Email all **error-free** text files and illustration jpgs **with a completed submission form** to <PennycookS@LandcareResearch.co.nz>. Place **first author surname + genus** + 'Mycotaxon' on the Email subject line; list title & coauthors and peer reviewer names & Email addresses in the message. The *Nomenclature Editor* will (i) reply immediately with the accession number and (2) return his notes with suggested revisions to the author(s) and *Editor-in-Chief* after his final review.

3—Final submission: All coauthors should consult experts and thoroughly revise and proof-read manuscripts to prepare error-free text and image files ready for immediate publication. **Label** the final submission email to the *Editor-in-Chief* <editor@mycotaxon.com> and **all** files & forms with **accession number;** attach the **(i)** 2018 submission form, **(ii)** labeled text files, **(iii)** labeled jpg files, and [required] **(iv)** FN, IF, or MB identification verification for each new name. The *Editor-in-Chief* usually contacts all coauthors and expert reviewers within two weeks of final submission, but please wait at least 14 days before sending a follow-up query (without attachments).

4—Final editorial review: Files with errors will be rejected or returned for revision. After completion of a thorough final grammatical and scientific review the *Editor-in-Chief* sends her editorial revisions to all coauthors and expert reviewers for final author approval prior to placement in the publication queue.

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Notes on some Japanese smut fungi. 6. *Macalpinomyces arundinellae-setosae* and *Sporisorium doidgeae* newly recorded, and new hosts of *Anthracoidea microsora*

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Abstract—*Macalpinomyces arundinellae-setosae,* known only from Australia and Thailand, is reported for the first time from Japan, on a new host plant, *Arundinella hirta. Sporisorium doidgeae* is also recorded as a new species for Japan, on *Capillipedium parviflorum. Carex pisiformis, C. sachalinensis* var. *iwakiana,* and *C. stenostachys* var. *stenostachys* are new host plants of *Anthracoidea microsora,* a species endemic to Japan.

Key words—*Anthracoideaceae, Cyperaceae, Poaceae,* taxonomy, *Ustilaginaceae*

Introduction

A taxonomic revision of specimens of smut fungi, as part of ongoing study of the smut fungi in Japan (Denchev & Kakishima 2000a, b, 2007; Denchev & al. 2000, 2006, 2011, 2013, 2015; Denchev & Denchev 2014), revealed two new fungal records for this country (*Macalpinomyces arundinellae-setosae* and *Sporisorium doidgeae*) and three new host plants of *Anthracoidea microsora: Carex pisiformis, C. sachalinensis* var. *iwakiana,* and *C. stenostachys* var. *stenostachys.*

Materials & methods

Specimens from the fungal dried reference collections of Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki (TSH), and the Kanagawa Prefectural Museum of Natural History, Odawara, Kanagawa (KPM), and infected dried plants from the Herbarium of Okayama University of Science, Okayama (OKAY) were examined under light microscope (LM) and scanning electron microscope (SEM). For LM observations and measurements, spores were mounted in lactoglycerol solution (w : la : gl = 1 : 1 : 2) on glass slides, gently heated to boiling point to rehydrate the spores, and then cooled. The spore measurements are given in the form: min–max (extreme values) (mean ± 1 standard deviation). For SEM, spores were attached to specimen holders by double-sided adhesive tape and coated with gold (in JEOL JSM-5510 and JEOL JSM-6390) or platinum-palladium (in JEOL JSM-7600F) in an ion sputter. The surface structure of spores was observed and photographed at 5 or 10 kV accelerating voltage using a JEOL JSM-5510 (Fig. 2), JEOL JSM-7600F (Fig. 5), and JEOL JSM-6390 (Figs 7–9) scanning electron microscopes. The height of warts was measured in SEM. The type of spore ornamentation, as seen by SEM, is in accordance with Denchev & al. (2013). The descriptions of smut fungi given below are based entirely on the specimens examined.

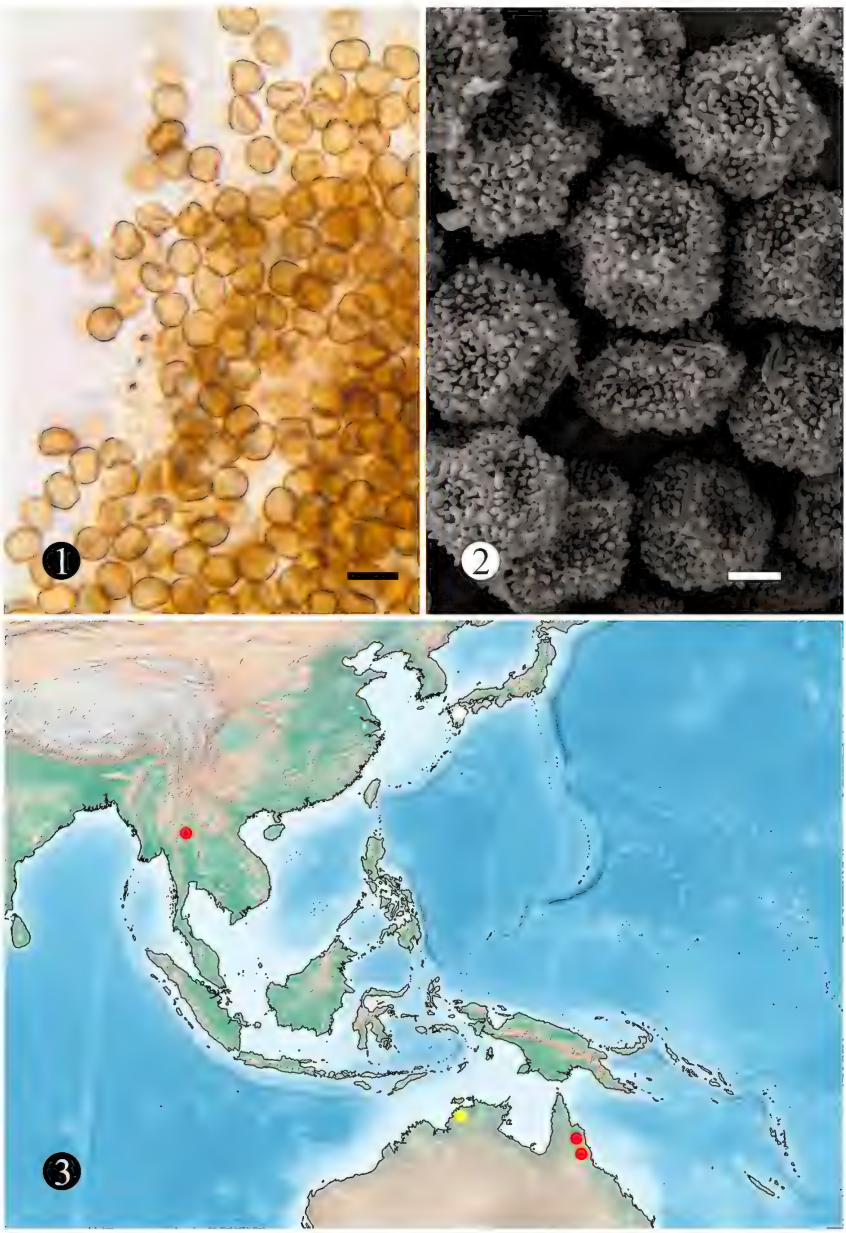
Taxonomy

Macalpinomyces arundinellae-setosae R.G. Shivas & Vánky, Mycol. Balcan. 2: 101, 2005. Figs 1, 2

Sori in some ovaries of the inflorescence, 3–8 × 0.5–0.8 mm, cylindrical, protruding beyond the spreading glumes; initially covered by a fragile, yellow-brown peridium that ruptures irregularly from its distal part, exposing the powdery, dark brown mass of spores and sterile cells. Sterile cells in irregular groups, 6–10 µm long, hyaline; wall 0.5–0.8 µm thick, smooth. Spores subpolyhedral, subglobose, broadly ellipsoidal, globose or ovoid, 6.5–9(–9.5) × 6–8(–9) µm (8.1 ± 0.6 × 7.3 ± 0.5 µm; n = 100), medium yellow-brown; wall more or less evenly thickened, 0.5–0.8 µm thick, in LM verruculose, spore profile usually slightly affected. In SEM moderately verruculose-echinulate, ornaments up to 0.4 µm high.

Specimen examined—On *Arundinella hirta* (Thunb.) Tanaka: **JAPAN, Kyushu, Kumamoto Prefecture,** Yamaga-shi, Kamoto, 3 November 1906, leg. K. Yoshino (TSH S-803, as *Sorosporium arundinellae* on *Arundinella anomala* Steud.).

Comments—*Sorosporium arundinellae* Syd. & P. Syd. was described from Japan on the same host plant, *Arundinella hirta* (Sydow & Sydow 1901), and was subsequently included in some taxonomic treatments of the smut fungi in Japan (e.g. Ito 1936, Kakishima 1982) and China (e.g. Wang 1964, Guo 1990). This species was reduced to a synonym of *Ustilaginoidea arundinellae* Henn.



Figs 1–3. ***Macalpinomyces arundinellae-setosae*** on *Arundinella hirta*. 1. Spores in LM; 2. Spores in SEM. Scale bars: 1 = 10 µm, 2 = 2 µm. 3. Geographic distribution of *Macalpinomyces arundinellae-setosae:* white circle – on *Arundinella hirta,* red circles – on *A. setosa,* yellow circle – on *A. nepalensis* (generated with SimpleMappr, Shorthouse 2010).

(*Clavicipitaceae*) by Ling (1951: 106 & 1953: 344; confirmed by Vánky 2011). We studied an isotype of *Sorosporium arundinellae* (Saitama Pref., Saitama-shi, Omiya, 20 November 1899, leg. T. Nishida, TSH S-4590) and confirm that it is not a smut fungus.

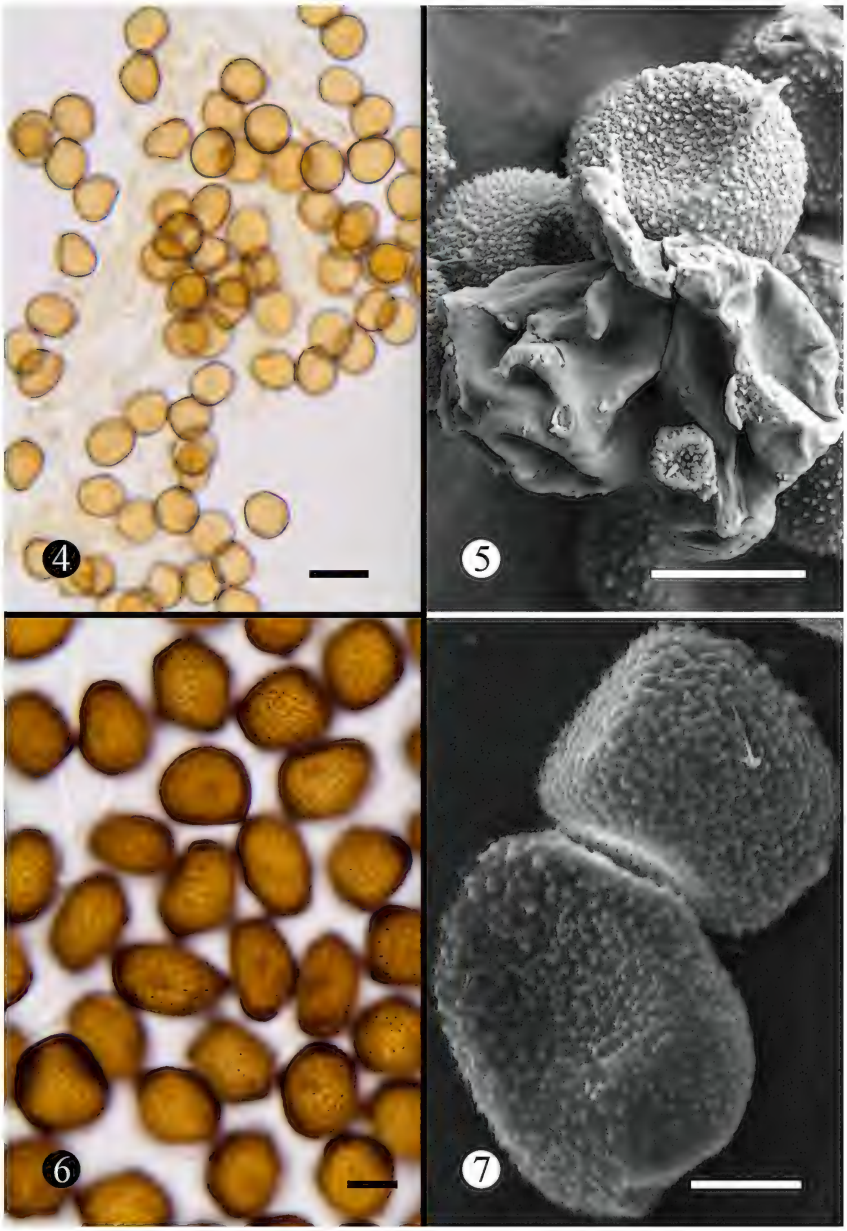
Macalpinomyces arundinellae-setosae is reported here for the first time from Japan, on a new host plant, *Arundinella hirta.* This smut fungus is known on *Arundinella setosa* Trin. from Queensland, Australia, and Thailand (Shivas & Vánky 2005, Shivas & al. 2007, Vánky & Shivas 2008), and on *A. nepalensis* Trin. from the Northern Territory, Australia (Vánky & Shivas 2008, Shivas 2010) (Fig. 3).

Sporisorium doidgeae (Zundel) Langdon & Full., Mycotaxon 6: 452, 1978. Figs 4, 5

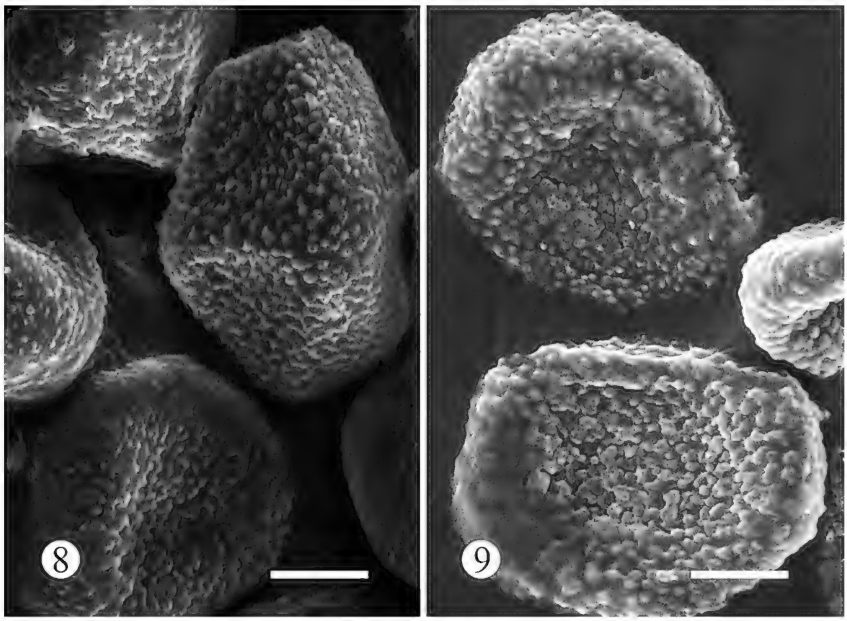
Sori variable, destroying the whole inflorescence, or some branches of the inflorescence, or some racemes, or groups of spikelets, or single spikelets. Accordingly, the sori can be cylindric, irregularly branched or compound, often with intact inflorescence branches or groups of spikelets; when destroying the whole inflorescence up to 4 cm long, partly concealed by the uppermost leaf sheath, when destroying single spikelets 1.5–2.5 × 0.7–1 mm; initially covered by a fragile, dark brown or ferrugineous brown peridium which later flakes away exposing a single or branching columella with shallow longitudinal furrows, surrounded by semi-agglutinated to powdery dark reddish brown mass of spores and sterile cells. Sterile cells in irregular groups or in chains, subglobose, collapsed, 7–11(–13) µm long, hyaline or subhyaline; wall 0.5–0.8 µm thick. Spores subglobose, globose, slightly irregular, broadly ellipsoidal or ovoid, rarely ellipsoidal, (7.5–)8–10(–11.5) × (7–)7.5–9.5(–10.5) µm (9.2 ± 0.6 × 8.3 ± 0.6 µm; n = 100), medium reddish brown; wall more or less evenly thickened, 0.5–0.7(–0.9) µm thick, minutely echinulate, spore profile not affected or very slightly affected. In SEM minutely echinulate, spinules up to 0.2(–0.3) µm high; densely punctate between the spinules.

Specimen examined—On *Capillipedium parviflorum* (R. Br.) Stapf: **JAPAN, Okinawa, Okinawa Prefecture,** Naha-shi, Shuri, 25 April 1953, leg. N. Hiratsuka (TSH S-3329, as *Ustilago* sp. on *Bothriochloa parviflora* (R. Br.) Ohwi).

Comments—Further information about smut fungi on *Capillipedium, Bothriochloa,* and *Dichanthium* is given in Vánky (2004) and Denchev & al. (2016). *Sporisorium doidgeae* is distributed in Asia (Pakistan, India, and China), Australia, and Africa (Ethiopia, Zimbabwe, and South Africa) (Vánky & Shivas 2008, Vánky 2011, Vánky & al. 2011). It is reported here for the first time from Japan.



Figs 4–7. ***Sporisorium doidgeae*** on *Capillipedium parviflorum*. 4. Spores and sterile cells in LM; 5. Spores and sterile cells in SEM. ***Anthracoidea microsora*** on *Carex pisiformis*. 6. Spores in LM; 7. Spores in SEM. Scale bars: 4, 6 = 10 µm, 5, 7 = 5 µm.



Figs 8, 9. ***Anthracoidea microsora*** spores in SEM. 8. On *Carex sachalinensis* var. *iwakiana;* 9. On *Carex stenostachys* var. *stenostachys.* Scale bars = 5 μm.

Anthracoidea microsora (Syd.) Kukkonen,
Ann. Bot. Soc. Zool.-Bot. Fenn. "Vanamo" 34(3): 55, 1963. Figs 6–9

Spores moderately verruculose to verrucose, warts 0.4–0.6 μm high, affecting the spore profile (for detailed description and illustrations of this species, see Denchev & al. 2013).

Specimens examined—On *Carex pisiformis* Boott (det. T. Katsuyama): **JAPAN, Honshu, Kanagawa Prefecture,** Odawara, Iryuda, 14 May 2010, leg. C. Akahori & S. Sasaki (KPM-NC 0017281, as *Anthracoidea caricis*); 25 May 2013, leg. K. Otsubo (pers. collection). —On *Carex sachalinensis* var. *iwakiana* Ohwi (det. H. Shimizu): **JAPAN, Honshu, Nagano Prefecture,** Kita-saku-gun, Miyota, Terasawa, 36°20′47″N 138°29′40″E, alt. 990 m, 10 June 2013, leg. H. Shimizu (OKAY-Shimizu2281). —On *Carex stenostachys* Franch. & Sav. var. *stenostachys* (det. T. Masaki): **JAPAN, Honshu, Okayama Prefecture,** Maniwa-shi, Sugatani, Utsukushi-mori, 35°08′04″N 133°41′05″E, alt. 636 m, 2 June 2013, leg. T. Masaki (OKAY-23849).

Comments—*Anthracoidea microsora* is endemic to Japan and known to infect the following sedges: *Carex alterniflora* Franch. var. *alterniflora* [≡ *C. sachalinensis* var. *alterniflora* (Franch.) Ohwi], *C. duvaliana* Franch. & Sav., *C. fernaldiana* H. Lév. & Vaniot [≡ *C. sachalinensis* var. *fernaldiana* (H. Lév. & Vaniot) T. Koyama], *C. stenostachys* var. *cuneata* (Ohwi) Ohwi &

T. Koyama, and *C. tenuinervis* Ohwi (Denchev & al. 2013). Three additional host plants, *C. pisiformis, C. sachalinensis* var. *iwakiana,* and *C. stenostachys* var. *stenostachys,* are reported here. A specimen reported by Akahori (2011) as *Anthracoidea caricis* on *Carex pisiformis* was re-examined and re-identified as *A. microsora.* The specimens examined here matched closely the description of *A. microsora* in the monograph of *Anthracoidea* in Japan (Denchev & al. 2013).

Acknowledgements

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Ascotricha microspora sp. nov. from Cayman Islands

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Abstract—A new hyphomycete species, *Ascotricha microspora* collected from Cayman Islands in the Caribbean, is described and illustrated. The asexual fungus differs from *Dicyma vesiculifera* by having smaller, colorless conidia. *Ascotricha rugispora* (≡ *Surculiseries rugispora*) and *A. funiculosa* (≡ *Dicyma funiculosa*) are proposed as new combinations.

Key words—ITS, microfungi, phylogeny, saprobe, *Xylariaceae*

Introduction

Ascotricha Berk. was proposed as a monotypic ascomycete genus (Berkeley 1838) typified by *A. chartarum* Berk. Boulanger (1897) typified *Dicyma* Boulanger by *Dicyma ampullifera* Boulanger, which he described as the conidial state of *Chaetomium zopfii* Boulanger [≡ *Ascotricha zopfii* (Boulanger) Peyronel], all names that were later synonymized with *Ascotricha chartarum* (Hawksworth 1971). Von Arx (1982) concluded that *Dicyma* is synonymous with *Hansfordia* S. Hughes, *Basifimbria* Subram. & Lodha, *Gonytrichella*, and *Puciola* De Bert. Nonetheless, some mycologists still regard *Hansfordia* and *Basifimbria* as distinct genera (Seifert & al. 2011). As the type species of *Dicyma* 1897 (*D. ampullifera*) and *Ascotricha* 1838 (*A. chartarum*) are

Table 1. ITS sequences of *Ascotricha microspora* and allied taxa used for phylogenetic analysis.

Taxon	Accession no.*	Substrate	Locality	ITS	Reference
Ascotricha bosei	CBS448.93 [T]	Date palm plantation soil	Iraq	KF893283	Cheng & al. 2015
Ascotricha chartarum	CBS234.97	Tropical rain forest soil	Papua New Guinea	KF893284	Cheng & al. 2015
	B4C	Oil on canvas painting	Nigeria	KX246915	Okpalanozie & al. 2016
	B4F	Oil on canvas painting	Nigeria	KX246917	Okpalanozie & al. 2016
Ascotricha erinacea	S98	*Dendrobium officinale*	China	KT224876	—
	CBS535.73	Elephant dung	East Africa	KF893285	Cheng & al. 2015
	CBS407.87	Paper and cardboard	Netherlands	KF893287	Cheng & al. 2015
	$OUCMBIII_{10}1069$	Sediment	China	KF893281	Cheng & al. 2015
	NBRC31999	Leaf of broad-leaved tree	Israel	03199901	—
	NBRC9841	Urethane foam	—	00984101	—
	NBRC33232	House dust	Japan	03323201	—
Ascotricha guamensis	NBRC9991 (IMI 82619)	Soil	—	999103	—
Ascotricha longipila	CGMCC 3.15229 ($OUCMB_{01}18$) [T]	Marine algae	China	KC503896	Cheng & al. 2015
Ascotricha lusitanica	S19	*Dendrobium officinale*	China	KT224797	—
	CBS462.70 [IT]	*Cicer arietinum* seed	Portugal	KF893289	Cheng & al. 2015
Ascotricha microspora	NHES L1706 [T]	Unidentified decaying leaf	Cayman Islands	MF805818	This study
Ascotricha parvispora	CGMCC 3.14995 ($OUCMBI_{11}0001$) [T]	Living *Enteromorpha linza*	China	JX014298	Cheng & al. 2015
Ascotricha sinuosae	CGMCC3.149965 ($OUCMBI_{12}1190$) [T]	Living *Colpomenia sinuosa*	China	JX014299	Cheng & al. 2015
	A1S5-10	Beach soil	Malaysia	KJ780756	—
	F8	Plant endophyte	Sudan	KR673905	—
Daldinia bambusicola	E40F	Agarwood tree	India	KY425743	—
Daldinia childiae	2-00219-2	*Platycladus orientalis* leaf	China	KT192354	—
Daldinia eschscholtzii	JMRC SF 11930	*Paphiopedilum exul* root	Thailand	KU304335	Barnes & al. 2016

Daldinia petriniae	H Knudsen s.n. (C)	*Corylus avellana*	Denmark	AF176970	Johannesson & al. 2000
Dicyma funiculosa	CBS124.80 [T]	Soil	Spain	KF893286	Cheng & al. 2015
	CBS323.86	Forest soil	Spain	AY908992	U'Ren & al. 2016
Dicyma pulvinata	414-3	—	—	LC177968	Unpublished
	KACC44502	—	—	LC177971	Unpublished
	CBS194.56	*Prunus persica* twig	Italy	AY908993	U'Ren & al. 2016
	LCC11	Giant panda vaginal secretions	China	KF986550	—
	5023	*Eucalyptus globulus* leaves	Spain	FR667996	Sánchez Márquez & al. 2011
	CBS254.59	*Lycopersicon esculentum* leaf	Netherlands	AY908995	Peláez & al. 2008
Hansfordia nebularis	CBS115626	*Quercus robur*	Italy	KF893290	Cheng & al. 2015
Hansfordia pulvinata	HMQAU130012	Tomato leaf?	China	KP772241	—
	KACC44498	Hyperparasitizing *Passalora fulva* on organic tomato plants	Korea	HM060587	—
	NBRC30211	*Lithocarpus edulis*	Japan	03021101	—
	NBRC6791	—	—	00679101	—
	NBRC9007	—	—	00900701	—
Lopadostoma turgidum	CBS 133207 [T]	*Fagus sylvatica*	Austria	NR132036	Jaklitsch & al. 2014
Podosordaria muli	WSP167 [T]	Mule dung	Mexico	GU324761	Hsieh & al. 2010
Surculiseries rugispora	NBRC33167 [T]	*Bruguiera gymnorhiza* leaf	Japan	LC146763	Okane & al. 2001
	NBRC33168	*Bruguiera gymnorhiza* leaf	Japan	03316801	Okane & al. 2001
Xylaria hypoxylon	ATCC42768 [T]	Wood	USA	AY327477	Platas & al. 2004

* [T] = ex-holotype; [IT] = ex-isotype.

ATCC = American Type Culture Collection, Manassas, United States; **BICC** = Biocon culture collection, India; **BCC** = BIOTEC Culture Collection, Thailand; **CBS** = Westerdijk Fungal Biodiversity Institute (formerly Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; **HMQAU** = Fungal Herbarium of Qingdao Agricultural University, Qingdao, China; IMI = IMI Fungarium, the Royal Botanic Gardens, Kew, UK; **KACC** = Korean Agricultural Culture Collection, South Korea; **MFLUCC** = Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **NBRC** = the NITE Biological Resource Center, Japan; **NFCCI** = the National Fungal Culture Collection of India; **NRRL** = The Agricultural Research Service Culture Collection, USDA, USA; **OUCMB** = Herbarium of Ocean University of China, Marine Biology, Shandong, China; **UAMH** = UAMH Centre for Global Microfungal Biodiversity, University of Toronto, Canada; **WSP** = Washington State University, Pullman, Washington, U.S.A.

synonyms (Hawksworth 1971), the generic names are likewise synonymous, with *Ascotricha* having priority as the earlier name.

Here we describe a new asexual species in *Ascotricha*, collected from Cayman Islands.

Materials & methods

The fungus was collected from fallen leaves and isolated on malt extract agar and incubated at 25°C. Conidiophores, conidiogenous cells and conidia were mounted in 85% lactic acid. A staining agent, 0.1% lacto-fuchsin, was used to observe conidiogenous cells and septation of colorless conidia. All microscopic observations were made under Nomarski differential interference contrast optics of Olympus BX40 microscope. Photomicrographs were taken with an Olympus Microfire digital camera. Measurements of the fungal structures were statistically analyzed with Microsoft Office Excel 2013 with 95% confidence interval of means. The results are presented as ranges calculated based on mean ± standard deviation with n = number of fungal structures measured. The holotype specimen is conserved in the Herbarium, Connecticut Agricultural Experiment Station, New Haven, U.S.A. (NHES).

DNA extraction and ITS sequencing procedures followed Li & al. (2008) as carried out by Gen Script (Nanjing) Co., Ltd. (Nanjing, China). The ITS sequence underwent BLAST analysis via the NBCI web site (www.ncbi.nlm.nih.gov/blast/) for sequence similarity searches and comparisons. Forty-six sequences representing *Ascotricha*, *Dicyma*, *Hansfordia*, and several genera of *Xylariaceae* and allied families were chosen for phylogenetic analysis (Table 1). Sequences are aligned with Guidance2 (mafft) (http://guidance.tau.ac.il/ver2/). *Lopatostoma tugidum* CBS 133207 was included as outgroup. The ITS sequence has been deposited in GenBank. Phylogenetic analysis was conducted using MEGA7 (Kumar & al. 2016). Bootstrap test was carried out with 1000 replicates.

Results

Our phylogenetic analysis distinguished *A. microspora* from other *Ascotricha*/*Dicyma* species and supported it as sister to *Surculiseries rugispora*. As can be seen in Fig. 1, *Ascotricha microspora*, *A. bosei*, *A. chartarum*, *A. erinacea* [= *Dicyma olivacea*], *A. guamensis*, *A. longipila*, *A. lusitanica*, *A. parvispora*, *A. sinuosae* [≡ *Hansfordia sinuosae*], *Dicyma funiculosa,* and *Surculiseries rugispora* group within the same clade (the /Ascotricha clade) with 97% support; the inclusion of the ex-type sequence of the monotypic genus *Surculiseries* within /Ascotricha suggests that it is congeneric with *Ascotricha*. Fig. 1 shows all *Dicyma funiculosa* sequences as monophyletic with 99% support and all sequences of *Ascotricha erinacea* [= *Dicyma olivacea*] as monophyletic with 98% support. However, the *Ascotricha chartarum* sequences are not monophyletic, with two sequences (B4C and B4F) grouping

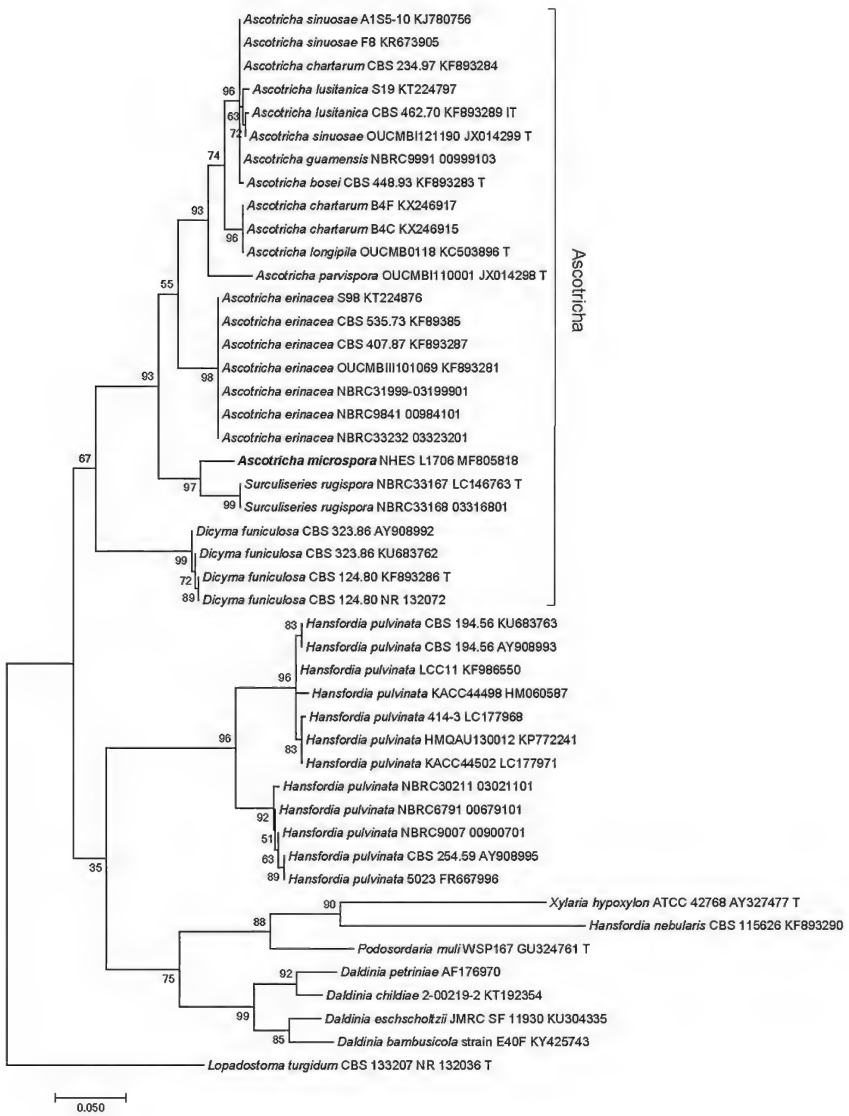

Ascotricha sinuosae A1S5-10 KJ780756
Ascotricha sinuosae F8 KR673905
Ascotricha chartarum CBS 234.97 KF893284
Ascotricha lusitanica S19 KT224797
Ascotricha lusitanica CBS 462.70 KF893289 IT
Ascotricha sinuosae OUCMBI121190 JX014299 T
Ascotricha guamensis NBRC9991 00999103
Ascotricha bosei CBS 448.93 KF893283 T
Ascotricha chartarum B4F KX246917
Ascotricha chartarum B4C KX246915
Ascotricha longipila OUCMB0118 KC503896 T
Ascotricha parvispora OUCMBI110001 JX014298 T
Ascotricha erinacea S98 KT224876
Ascotricha erinacea CBS 535.73 KF89385
Ascotricha erinacea CBS 407.87 KF893287
Ascotricha erinacea OUCMBIII101069 KF893281
Ascotricha erinacea NBRC31999-03199901
Ascotricha erinacea NBRC9841 00984101
Ascotricha erinacea NBRC33232 03323201
Ascotricha microspora NHES L1706 MF805818
Surculiseries rugispora NBRC33167 LC146763 T
Surculiseries rugispora NBRC33168 03316801
Dicyma funiculosa CBS 323.86 AY908992
Dicyma funiculosa CBS 323.86 KU683762
Dicyma funiculosa CBS 124.80 KF893286 T
Dicyma funiculosa CBS 124.80 NR 132072
Ascotricha
Hansfordia pulvinata CBS 194.56 KU683763
Hansfordia pulvinata CBS 194.56 AY908993
Hansfordia pulvinata LCC11 KF986550
Hansfordia pulvinata KACC44498 HM060587
Hansfordia pulvinata 414-3 LC177968
Hansfordia pulvinata HMQAU130012 KP772241
Hansfordia pulvinata KACC44502 LC177971
Hansfordia pulvinata NBRC30211 03021101
Hansfordia pulvinata NBRC6791 00679101
Hansfordia pulvinata NBRC9007 00900701
Hansfordia pulvinata CBS 254.59 AY908995
Hansfordia pulvinata 5023 FR667996
Xylaria hypoxylon ATCC 42768 AY327477 T
Hansfordia nebularis CBS 115626 KF893290
Podosordaria muli WSP167 GU324761 T
Daldinia petriniae AF176970
Daldinia childiae 2-00219-2 KT192354
Daldinia eschscholtzii JMRC SF 11930 KU304335
Daldinia bambusicola strain E40F KY425743
Lopadostoma turgidum CBS 133207 NR 132036 T
0.050

Fig. 1. Maximum Likelihood analysis of *Ascotricha microspora* and allied taxa, based on 46 sequences. *Lopatostoma tugidum* CBS 133207 is included as outgroup. The bootstrap test was conducted with 1000 replicates; bootstrap values >50% are indicated at the nodes. The scale bar indicates the number of expected changes per site. Annotations T and IT indicate the ex-type sequences included in the analysis.

with *A. longipila* while the third (CBS234.97) groups with *Ascotricha bosei*, *A. guamensis*, *A. lusitanica*, and *A. sinuosae* [≡ *H. sinuosae*].

All 12 isolates of *Hansfordia pulvinata* [≡ *Dicyma pulvinata*] were monophyletic with 96% support and sister to a clade (containing sequences of *Daldinia* and other genera) within which *H. nebularis* grouped with *Xylaria hypoxylon* (Fig. 1). The clear separation between these clades and the /Ascotricha clade indicates that *H. pulvinata* and *H. nebularis* do not belong in *Ascotricha*.

Taxonomy

Ascotricha Berk., Annals of Natural History 1: 257 (1838)

= *Dicyma* Boulanger, Revue Générale de Botanique 9: 18 (1897)

= *Gonytrichella* Emoto & Tubaki, Transactions of the Mycological Society of Japan 11: 95 (1970)

= *Puciola* De Bert., Mycotaxon 3(3): 553 (1976)

= *Surculiseries* Okane, Nakagiri & Tad. Ito, Mycoscience 42(1): 116 (2001)

Ascotricha microspora D.W. Li & G.H. Zhao, **sp. nov.** Fig. 2

MycoBank MB 822539

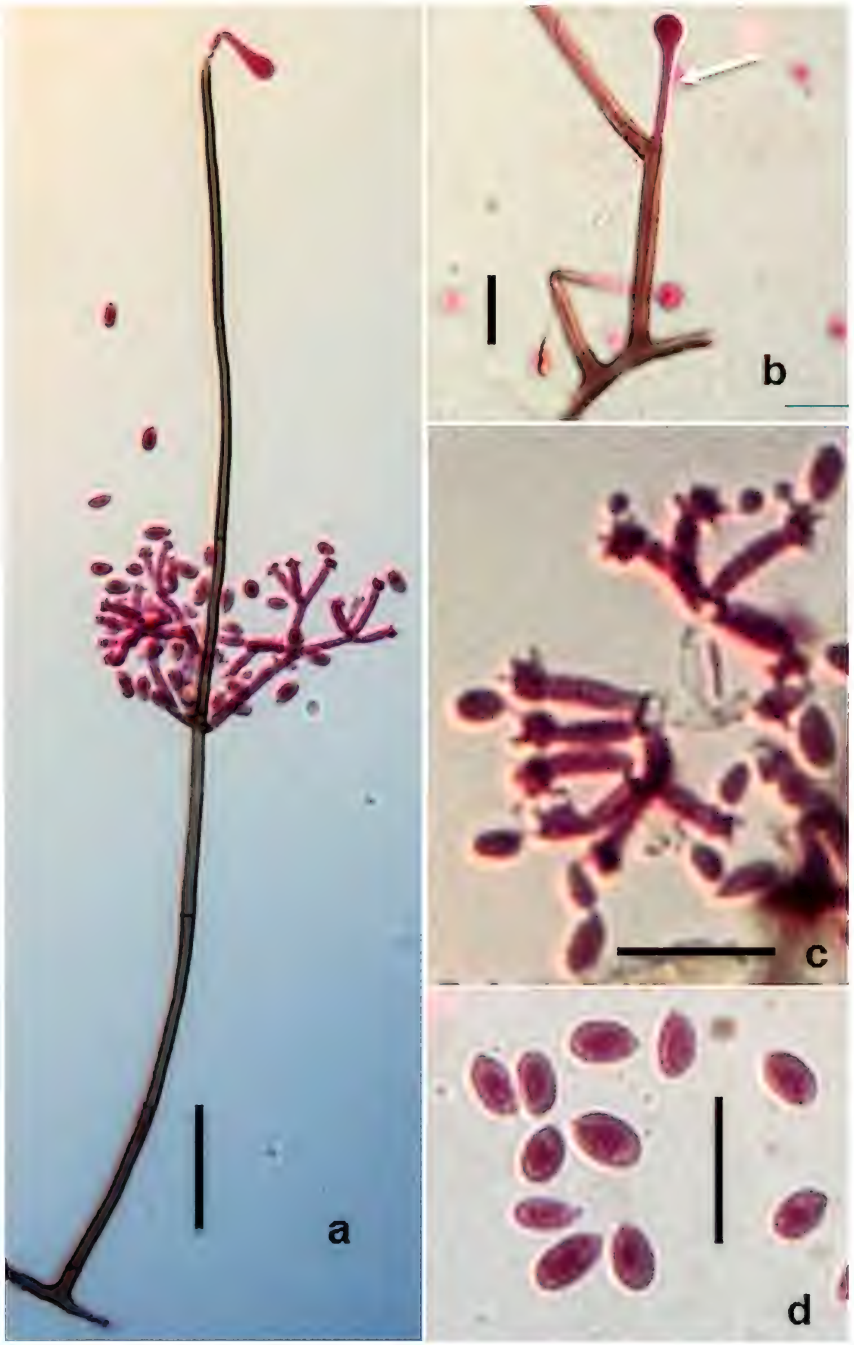
Differs from *Dicyma vesiculifera* by its smaller, colorless conidia.

Type: Cayman Islands, Grand Cayman, fallen leaves, 2 January 2012, De-Wei Li (**Holotype**, NHES L1706 (GenBank MF805818).

Etymology: epithet means small conidia.

Colonies hypophyllous, effused, lanose, gray. Mycelium partly superficial, partly immersed in the substrate, composed of colorless to pale brown hyphae, 1–2 µm diam. Conidiophores differentiated, simple, solitary, ≤220 µm long, 2.5–3.2 µm diam, smooth, erect or geniculate, alternately branched, brown to pale brown, gradually tapering toward the apex, septate, usually terminating in a capitate or spathulate cell and often developing a similar cell at each geniculation. The capitate or spathulate cells (drumstick cells) are sterile, colorless, smooth, thin-walled, (10.8–)12–15.4(–17.1) × (1.3–)1.4–2(–2.4) µm (mean ± SD = 13.7 ± 1.6 × 3.8 ± 0.6 µm, n = 12), with an apex enlarged into globose or subglobose shaped vesicle, (2.9–)3.2–4.4(–4.9) µm diam. (mean ± SD = 3.8 ± 0.6 µm, n = 12). Lateral branches arising singly, in pairs or in whorls, penicillate, colorless or pale brown below and colorless above, thin walled, smooth, up to 50 µm long, sometimes developing a thin-walled

Fig. 2. *Ascotricha microspora* (holotype, NHES L0706). a. Conidiophore, conidiogenous cells, and conidia; b. Partial conidiophore and a drumstick cell at geniculation (arrowed); c. Conidiogenous cells and conidia; d. conidia. Scale bars: a = 20 µm; b–d = 10 µm.


a
b
c
d

ovoid or pyriform, colorless vesicle laterally just below secondary branches, (4.6–)5.3–7.8(–8.7) × (2.5–)3–5(–6) µm, (mean ± SD = 6.6 ± 1.2 × 4 ± 1 µm, n = 10). Conidiogenous cells polyblastic, cylindrical, colorless, smooth, thin-walled, (5–)6–8(–10) × (1.3–)1.4–1.7(–1.9) µm, (mean ± SD = 7 ± 1 × 1.57 ± 0.16 µm, n = 21), denticulate, enlarged at the apex bearing a cluster of 3–6 denticles, 0.5–1.4 × 0.5–0.9 µm; conidial secession schizolytic. Conidia solitary, ovoid or ellipsoid, rounded at the apical end, with a papilate basal end, unicellular, smooth, hyaline, (3.7–)4.2–4.8(–5) × (2.1–)2.4–2.8(–3) µm, (mean ± SD = 4.5 ± 0.3 × 2.6 ± 0.2 µm, n =30).

Discussion

Ascotricha microspora is morphologically rather similar to *Dicyma vesiculifera* Piroz. but differs in conidial color and size, with *D. vesiculifera* distinguished by pale yellow-brown and longer (5–7 µm) conidia; Pyrozynski 1972). Both species produce conidiogenous cells that are enlarged significantly at the apical end and bear denticles. Conidiogenesis in both species is somewhat similar to that found in *Hansfordia*, and their conidiophores develop thin-walled, colorless capitate/spathulate cells that resemble those in *Dicyma*. Thus, *Ascotricha microspora* and *D. vesiculifera* have a morphology intermediate between those in typical *Dicyma* and *Hansfordia* species.

Surculiseries was erected and typified with *S. rugispora* based on its polyblastic, backward-curving conidiogenous cells with conspicuous scars on the flattened denticle tops and ITS and LSU sequence analysis (Okane & al. 2001). *Surculiseries* remains monotypic but shares many morphological characters with *Ascotricha* [= *Dicyma*]. However, *S. rugispora* differs from *A. microspora* in conidial morphology and conidiogenous cells. The phylogenetic analysis of Okane & al. (2001) supports *Surculiseries* as paraphyletic to *Ascotricha* and its asexual state, *Dicyma*. However, our ITS-based sequence analysis supports *Ascotricha* and *Surculiseries* as congeneric (Fig. 1). LSU and SSU phylogenies generated from using available GenBank and NBRC sequences confirm the results of the ITS phylogenetic analysis (unpublished data). We therefore propose a new combination of *S. rugispora* in *Ascotricha:*

Ascotricha rugispora (Okane, Nakagiri & Tad. Ito)
D.W. Li & G.H. Zhao, **comb. nov.**
MycoBank MB 822541
≡ *Surculiseries rugispora* Okane, Nakagiri & Tad. Ito, Mycoscience 42(1): 121 (2001)

Our phylogenetic analysis (Fig. 1) also supports *Dicyma funiculosa* in the /Ascotricha clade. Consequently, we propose a new combination of *Dicyma funiculosa* in *Ascotricha:*

Ascotricha funiculosa (Guarro & Calvo) D.W. Li & G.H. Zhao, **comb. nov.**

MycoBank MB 824983

≡ *Dicyma funiculosa* Guarro & Calvo, Nova Hedwigia 37(4): 641 (1983)

The type species of *Hansfordia* is *H. ovalispora* S. Hughes (Hughes 1951), with no available culture or sequences. The phylogenetic relationship between *Dicyma* and *Hansfordia* remains unresolved. That is also the case with *Basifimbria aurea* Subram. & Lodha, the type species of *Basifimbria* (Subramanian & Lodha 1968). Since there are no sequence data of *Basifimbria* available, we cannot establish whether *Basifimbria* is congeneric with *Dicyma* or *Hansfordia*. Further studies are necessary to determine the phylogenetic relationships among the three genera.

Ascotricha sinuosae, *A. lusitanica, A. guamensis*, and *A. bosei* (together with one sequence labelled as "*A. chartarum*") share the same clade, with 96% support. Potential synonymies cannot be resolved because of the lack of support within this clade, and the delineation of the three species needs to be further studied with multiple genes.

The current phylogenetic analyses suggest that *Hansfordia pulvinata* and *H. nebularis* may not belong to *Ascotricha* (Fig. 1), and their placement is yet to be determined.

Acknowledgments

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Tolypocladium dujiaolongae sp. nov. and its allies

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Abstract—A common cicada pathogen occurring in bamboo forests of southern China is described as a new species, *Tolypocladium dujiaolongae*. The previously unknown anamorphic state was obtained from discharged ascospores. Based on its morphological characteristics and a molecular analysis of the internal transcribed spacer rDNA sequence, we found that the asexual features of *T. dujiaolongae* are similar to those of *Tolypocladium* species. The typical characteristics of the fungus are described. The fruiting bodies formed on artificial culture media were identical to those of natural specimens, strongly supporting the idea that the isolated strain is the asexual stage of *T. dujiaolongae*. Our results indicate that there is no relationship between *Tolypocladium dujiaolongae* and the famous traditional Chinese medicine *Isaria cicadae*.

Key words—anamorphic–teleomorphic connection, morphology, phylogeny, mycoparasite, taxonomy

Introduction

The use of cordycipoid pathogens of cicadas in Traditional Chinese Medicine (TCM) has a longer documented history (>1500 years) than the TCM usage of *Ophiocordyceps sinensis* (Berk.) G.H. Sung & al. (c. 500 years). The adjective "cordycipoid" refers to all taxa in the families *Clavicipitaceae*, *Cordycipitaceae*, and *Ophiocordycipitaceae*. Use of the cicada pathogen can be traced to a 5th

century medical work by Leixiao, titled "Lei's Treatise on Preparing Drugs" (雷公炮炙论), which recorded the preparation of a cicada pathogen, referred to as "cicada flower" (Chan-hua 蝉花) for medical use against childhood disorders. This "cicada flower" has generally been identified as *Isaria cicadae* Miq. (Fig. 1). In the market a small proportion of specimens appear more robust leading to the Chinese folk recognition of a "male (robust) cicada flower" as compared to the "female cicada flower" with its slender synnemata. In southern China, the "male" form is more often called the "single horned dragon" (Dujiaolong, 独角龙) due to its solitary robust stroma.

Shing (1975) reviewed these wild collections and recognised the "male cicada flower" as the teleomorph, which he mistakenly associated with *I. cicadae*. As well as making this link he also distinguished between *Isaria cicadae* and *Cordyceps sobolifera* (Hill ex Watson) Berk. & Broome, which is also sold and named as "cicada flower" in some areas in southern China.

Approximately 30 species of *Cordyceps* sensu lato and related genera are known to be pathogens of cicadas. Of these, *C. sobolifera*, *I. cicadae*, and *Cordyceps cicadae* (Miq.) Massee, are three New World names that have been widely used in the Old World (especially east Asia) without real reference, or comparison, to the type materials. Following the work of Sung & al. (2007), *C. sobolifera* was transferred to the genus *Ophiocordyceps* in the newly created family *Ophiocordycipitaceae*. The latter two taxa retained the same names within the family *Cordycipitaceae*. Historically, *Ophiocordyceps sobolifera* was the earliest named cordycipoid cicada pathogen (CCP), based on material from Guadeloupe and other islands of the Caribbean (Watson 1763). *Isaria cicadae* was later described by Miquel (1838) from a cicada in Brazil. The name *Cordyceps cicadae* was proposed for Miquel's taxon by Massee (1895) on the assumption that the *Isaria* was the 'imperfect' form of an unknown *Cordyceps*; however, no teleomorphic *Cordyceps* state has yet been described for a South American *I. cicadae*.

Throughout the twentieth century these three names were frequently applied to east Asian taxa of CCPs: with many collections from China, Japan, Korea, and Thailand, as well as New Zealand. Petch (1924, 1931, 1933, 1935, 1942) reviewed these names, as did Kobayasi (1939, 1941). Although Petch (1933) did not link *I. cicadae* and *C. sobolifera* in a list of synonyms, he later (Petch 1935, 1942) accepted *I. cicadae* as the anamorph of *C. sobolifera*. This link was also accepted by Mains (1958). Significantly, while Petch and Mains were not familiar with these fungi in the field, Kobayasi (1941) demonstrated that *I. cicadae* and *C. sobolifera* (as understood from Asian material) were unrelated.

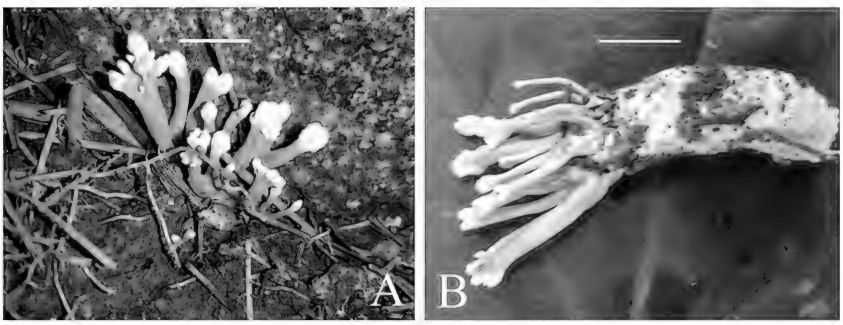

FIG. 1 Chan-hua (*Isaria cicadae*).
A: Emergent synnemata. B: Excavated Chan-hua. Scale bars = 10 mm.

In China, Shing (1975) recognised *C. sobolifera* and *I. cicadae* as distinct species based on Chinese collections of the two taxa although he accepted that the Asian *C. sobolifera* had an *Isaria* anamorph. Seemingly unaware of the Massee (1895) combination for the South American material, Shing (1975) further described a *Cordyceps* teleomorph that he associated with the Asian *I. cicadae*, and named this *Cordyceps cicadae* S.Z. Shing (an illegitimate later homonym of the Massee name). This anamorph–teleomorph link was based on the temporal and spatial co-occurrence of the two taxa in bamboo forest habitats in southern China and the proximity of the teleomorph specimens to collections of *I. cicadae* and to their being sold together in markets.

In 2012 and 2013, we collected specimens of CCPs from the Guniujiang Nature Preserve (Anhui Province, southeastern China). This is an area where medicinally valuable cicada 'cordyceps' have traditionally been collected. Our anamorph collections matched the Asian concept of *I. cicadae,* while our teleomorph specimens matched Shing's *C. cicadae*. However, isolates derived from ascospores produced an anamorph in culture that was not an *Isaria* (as had been concluded by Shing 1975). Rather, it belonged to the genus *Tolypocladium* based on morphological characteristics and a comparison of its 5.8S rDNA and ITS sequences. This *Tolypocladium* species is described here.

Materials & methods

Specimens and fungal isolates

Twenty-one specimens of CCPs were collected in bamboo forest from the Guniujiang Nature Preserve (Qimen County, southern Anhui, China, 30°01′N

117°31′E) at an elevation of approximately 420 metres above mean sea level. The specimens' hosts were soil-dwelling nymphs of an unidentified cicada. Isolates were derived from ascospores discharged onto glass slides according to the method of Li & al. (1999), RCEF6201 from GNJ130616-01 (= ZBAH632) and RCEF6202 from GNJ130617-02 (= ZBAH633). Many specimens of *I. cicadae* were also collected and isolated. One isolate of *I. cicadae* (RCEF0817-13) was further used in this study.

All specimens were freeze-dried and stored at 4°C after isolation and description as preparation for DNA extraction. The morphological characteristics of cultures incubated on potato dextrose agar (PDA) and Czapek-Dox agar at 25°C for 7 and 14 days were recorded. Specimen vouchers were conserved in the herbarium of Zhejiang BioAsia Pharmaceutical Co, Ltd., Zhejiang, China (ZBAH); and strains were conserved in the culture collection of Research Center on Entomogenous Fungi, Hefei, China (RCEF).

DNA extraction, PCR, and sequencing

Fresh mycelium (RCEF6201 and RCEF0817-13) was obtained from the surface of PDA plates that had been incubated at 25°C for 14 days. Approximately 0.1 g of sample was ground under liquid nitrogen in 1.5 ml Eppendorf tubes, and genomic DNA extracted using benzyl chloride according to a modification of the method described by Zhu & al. (1994).

The PCR mixtures, totalling 25 µL, were composed of 2.5 µL of 10 × Buffer (10 mM Tris/HCI, pH 8.3), 0.5 µL of a mixture containing 10 mM each of the four deoxyribonucleotide triphosphates (dNTP), 1.0 µL of each primer at 10 µmol/L (ITS4: 5′-TCCTCCGCTTATTGATATGC-3′ and ITS5: 5′-GGAAGTAAAAGTCGTAACAAGG-3′, Sango Biotech Co., Ltd., Shanghai), 0.2 µL of 5 U/µL DreamTaq polymerase (Fermentas, Thermo Scientific), 1 µL of template DNA, and 18.8 µL of double-distilled water.

The PCR was performed under the following temperature profile: 95°C for 5 min, followed by 37 cycles of 95°C for 30 s, 56°C for 30 s, and 72°C for 50 s, and a final extension at 72°C for 10 min. The PCR products were detected on an ethidium bromide (EB) gel (1.0% agarose gel including 0.5 g/ml EB).

The purification of the resulting products, as well as the sequencing of forward and reverse strands, was performed at the Beijing Genomics Institute (BGI). The sequencing data for the Anhui CCP (teleomorph and anamorph) and *I. cicadae* were submitted to GenBank.

Alignments and analysis

ITS1-5.8S-ITS2 sequences and those that we downloaded from GenBank were imported into the programme BioEdit Sequence Alignment Editor Version 4.8.6., aligned using Clustal X 2.0 for multiple sequence alignment and corrected manually (Hall 1999). The phylogenetic tree was constructed by the Neighbor-joining method (NJ) of MEGA 4.0 with 1000 bootstrap replicates. *Beauveria bassiana* (AF347611) was used as an outgroup taxon in the analyses. Bootstrap support of ≥70% was superimposed on all tree constructions.

Results

Molecular results

The ITS1-5.8S-ITS2 region of *I. cicadae* (GenBank KF740422) comprised 498 bp and showed a similarity of only 75.6% with the Anhui CCP. Both PCR products of the field-collected teleomorph and the culture of the Anhui CCP were detected by agarose gel electrophoresis. The amplified fragments spanning the ITS1-5.8S-ITS2 of the teleomorphic field material (GenBank KF696557) and the anamorphic culture (GenBank KF696558) were the same size (485 bp). The sizes of ITS1 (165 bp), 5.8S rDNA (146 bp), and ITS2 (174 bp) regions were also identical. This evidence confirmed that the isolate RCEF6201 was the anamorph of the field material ZBAH632, and not the *Isaria* anamorph of *C. cicadae* as concluded by Shing (1975).

To determine the phylogenetic position of the Anhui CCP, the sequences of the field material and the ex-ascospore strain RCEF6201 were compared with available sequences in GenBank via a BLAST search. These sequences revealed the Anhui CCP to be linked with the genus *Tolypocladium* (Fig. 2) and not with *Cordyceps cicadae* / *Isaria cicadae* as described by Shing (1975). However, the sequences and morphologies of the Anhui CCP and its cultured anamorph differed from those of other *Tolypocladium* spp.

Our study of the Anhui CCP revealed a new clade and suggested that it is a distinct taxon in *Tolypocladium* based on the molecular and morphological information. The teleomorph and culture of the CCP clustered in one group strongly supported by bootstrap proportions (BP = 100%). A sister-group relationship between *Tolypocladium paradoxum* (Kobayasi) C.A. Quandt & al. and the Anhui CCP was also strongly supported (BP = 100%) (Fig. 2).

BLAST searches in GenBank suggested that there are several *Tolypocladium* species similar to the Anhui CCP. The Anhui CCP shared 98.1% homology with *T. paradoxum* (AB027369), which is also pathogenic on cicada nymphs, and 94.9% with *Tolypocladium ophioglossoides* (J.F. Gmel.) C.A. Quandt & al. (AJ309360), which is pathogenic on *Elaphomyces*. It also shared a high level of ITS sequence identity with a sequence from China identified as *Cordyceps imagamiana* Kobayasi & Shimizu. Although there was little molecular divergence between the Anhui CCP and *C. imagamiana*, the morphological differences between the Anhui CCP and the original Japanese description of *C. imagamiana* were noticeable (Kobayasi & Shimizu 1983, Shimizu 1997 Plate 23). On the basis of these results we describe the Anhui CCP as a new species of *Tolypocladium* (sensu Quandt & al. 2014) in accordance with the one fungus = one name (1F = 1N) principle.

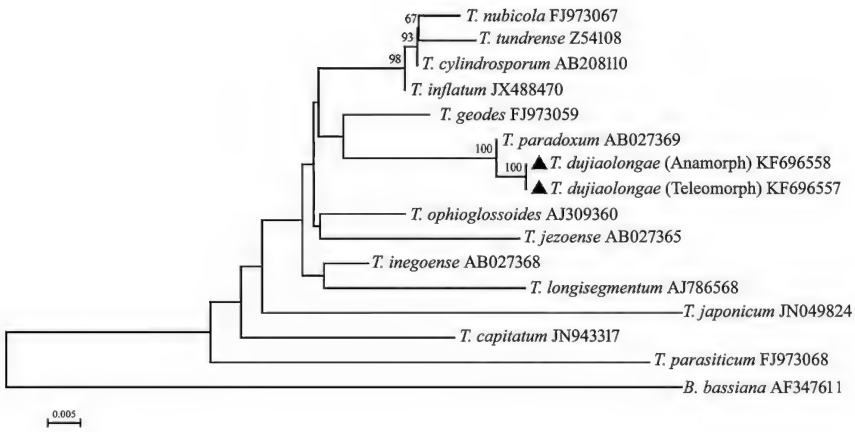

Fig. 2 Neighbor-joining tree based on ITS1-5.8S-ITS2 region sequence data from *Tolypocladium dujiaolongae* and related species. Values above the branches indicate bootstrap support.

Taxonomy

Tolypocladium dujiaolongae Y.P. Cao & C.R. Li, **sp. nov.** Figs 3, 4

MycoBank MB821894

Differs from *Cordyceps cicadae* S.Z. Shing by its larger perithecia and larger asci; and from *C. imagamiana*, *Tolypocladium inegoense*, and *T. paradoxum* by its blackish-brown to blackish-purple stipe, its brown to black fertile parts, its larger perithecia, and stipe with or without rhizoids attached to the host.

Type: China. Anhui Province: Qimen County, National Natural Reserve of Guniujiang, 30°01′N 117°31′E, alt. 420 m, 16.VI.2013, Y.P. Cao GNJ130616-01 (**Holotype**, ZBAH632; GenBank KF696557; ex-ascospore culture, RCEF6201, GenBank KF696558).

Etymology: *dujiaolongae* refers to the traditional Chinese name for the species, which translates as "single-horned dragon".

Stroma arising from the head of the nymphal cicada host, blackish brown to blackish-purple, fleshy, mostly solitary, occasionally branched, erect or curving, 26–70 (rarely 105) mm long, clavate or obclavate, corniform or oblate, with a blunt or slightly apiculate and tuberculate tip and sunken grooves. Stipe cylindric, 5–10 mm thick, 10–45 mm long, brown or yellow. Fertile part clavate, 40–55 × 4–12 mm, clearly defined from the stipe, punctate with perithecial ostioles, brown to black, without sterile tip. Perithecia ampullaceous, wholly immersed, (233–)520–740(–780) × (250–)300–330

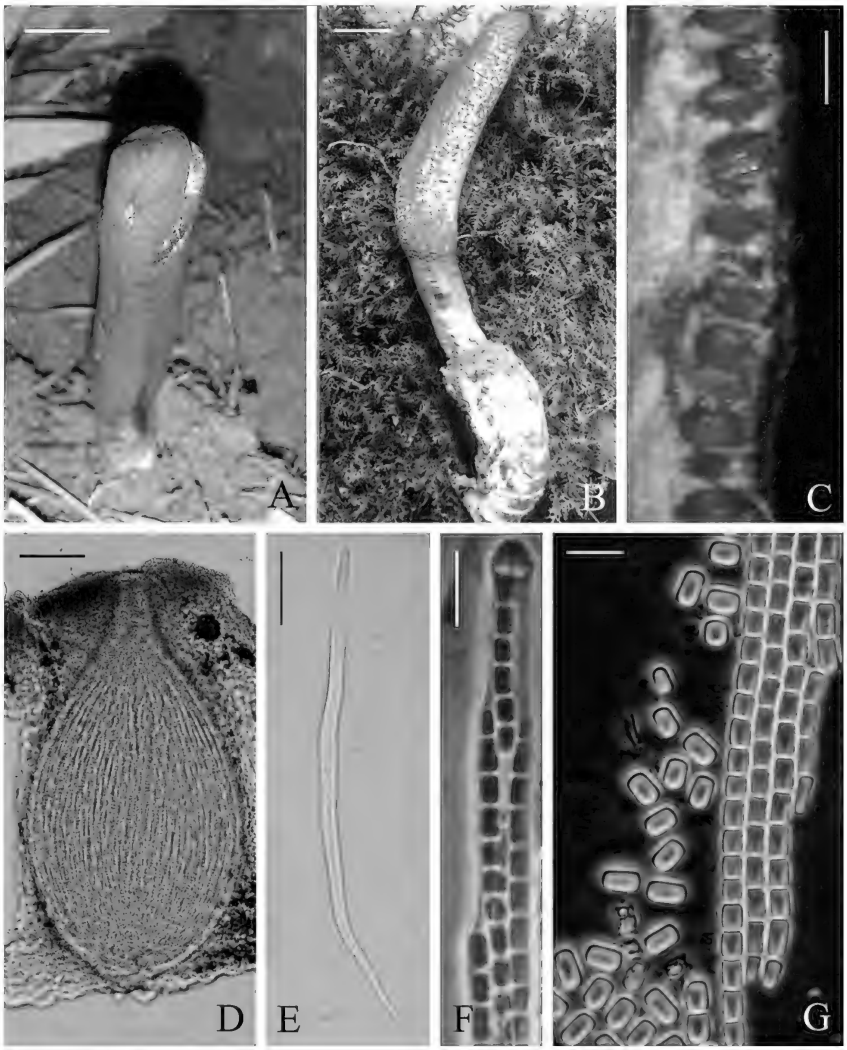

Fig. 3 *Tolypocladium dujiaolongae* teleomorph. A, B: Stroma of *Tolypocladium dujiaolongae*; C: Wholly immersed perithecia; D: Perithecium; E: Whole ascus; F: Upper part of ascus; G: Part spores. Scale bars: A, B = 10 mm; C = 500 µm; D = 100 µm; E = 50 µm; F, G = 10 µm.

(–360) µm, with wall 23.0–33.5 µm thick and ostioles 34–80 µm in diameter. Asci 8-spored, hyaline, cylindrical, (380–)420–468(–500) × 8–11 µm. Ascus cap hemispherical, 5.5–9.0 µm in diameter, 4.0–8.0 µm high. ascospores hyaline, filiform, 240–310 µm long when discharged, smooth, multiseptate,

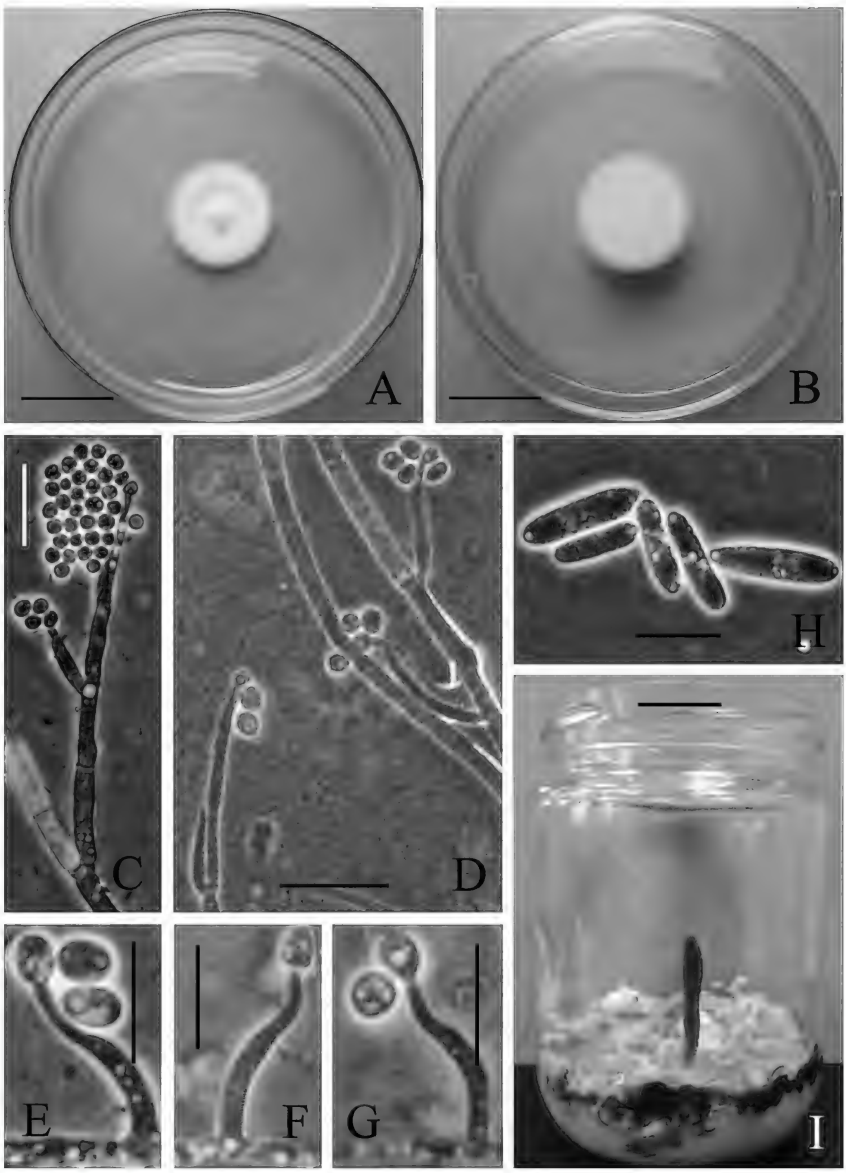

Fig. 4 *Tolypocladium dujiaolongae* anamorph. A: Colony on PDA (25°C, 14 d); B: Colony, reverse side; C–H: Conidiophores and conidia; I: Stroma of *Tolypocladium dujiaolongae* on artificial media. Scale bars: A, B = 2 cm; C–H = 10 μm; I = 20 mm.

breaking into cylindrical part spores, 3–5(–7.0) × 2–3 µm, germinating at 25°C on PDA.

Colonies growing slowly, 10–15 mm in diameter after 7 days on Czapek-Dox agar at 25°C; velutinous, loose, white to yellowish, hemispherical with a 2–3 mm high region protruding in the colony centre. Reverse white. Colonies on PDA growing moderately at 25°C after 7 days, 16.0–18.0 mm diam., and 28–30 mm after 14 days on PDA, velutinous, loose, white. Reverse centre yellow-orange to dark brownish, with white margin. Hyphae septate, smooth-walled, hyaline, 2.0–3.5 µm wide. Phialides solitary, occasionally verticillate, (5–)11–35(-52) × 1.0–2.7 µm, usually growing on the aerial mycelium or on simple conidiophores, cylindrical or conical, slightly swollen basally, narrowly tapering into a distinct neck approximately 1.0 µm wide, terminal phialides generally elongate; neck often bent without clear denticulate scars. Conidia one-celled, hyaline, smooth-walled, globose to ovoid when separate, and polyhedral when aggregated, (2.5-)3–4(–4.7) × 2.4–3.7 µm, aggregating mostly in small heads, 11.0–36.8 µm in diameter and occasionally in chains at the tips of the phialides; a very small proportion of the conidia much larger, long ellipsoidal, approximately 5–9 µm wide and up to 8–25(–36) µm long.

Ecology & distribution—Scattered in bamboo forest. Summer. Common in southern China, especially in Zhejiang, Anhui, Jiangsu, Jiangxi, and Fujian provinces.

Additional specimen examined — China. Anhui Province: Qimen County, National Natural Reserve of Guniujiang, 30°01′N 117°31′E, alt. 420 m, 16.VI.2013, Y.P. Cao GNJ130617-02 (ZBAH633; ex-ascospore culture RCEF6202).

Discussion

Taxonomy and phylogeny

The macroscopic and microscopic characteristics of *T. dujiaolongae* specimens collected from the Guniujiang Nature Preserve were nearly identical to those described as *C. cicadae* by Shing (1975), especially with regards to the shape and size of the part spores but with exceptions for the larger perithecia and longer asci (Table 1). The teleomorphs of *T. paradoxum*, *Tolypocladium inegoense* (Kobayasi) C.A. Quandt & al., and *C. imagamiana* were compared with *Tolypocladium dujiaolongae* (Table 1, Fig. 2). These are all parasitic on cicada nymphs and produce cylindrical part spores. Although the ITS sequences were similar there are obvious differences between *T. paradoxum* and *T. dujiaolongae* in the appearance of their stromata. Those of *T. paradoxum* are pale purple and attached to the host by subterranean whitish rhizoids

(Shimizu 1997, Plate 24). In comparison, the stroma of *T. dujiaolongae* is brown or yellow and simple attached directly to the host without rhizoids.

Tolypocladium inegoense is easily distinguished from *T. dujiaolongae* by the superficial (but crowded) nature of its perithecia compared with the immersed form of *T. dujiaolongae* and the colour of its fertile part which is a dark olive green compared with the blackish brown of *T. dujiaolongae* (Shimizu 1997 Plate 14). According to the description and the picture of *C. imagamiana* (Kobayasi & Shimizu 1983; Shimizu 1997 Plate 23), *T. dujiaolongae* and *C. imagamiana* are also quite different in the appearance of their stromata. The latter have longer stromata (90 mm) and a thinner stipe (1.5–2.0 mm) with a pallid ochre and oblong fertile part. We have no further information regarding the specimen identified as *C. imagamiana* from China and consider this named to have been misapplied.

The morphological features of the cultured anamorph of *T. dujiaolongae* are also consistent with the genus *Tolypocladium*. Four species in this genus (*T. inflatum* W. Gams, *T. cylindrosporum* W. Gams, *T. geodes* W. Gams, and *T. tundrense* Bissett) are similar to the anamorph of *T. dujiaolongae*. The conidiogenous cells of *T. dujiaolongae* are similar in shape and size to those of *T. geodes*, but this species differs in only having one type of conidia, either subglobose or broadly obovoid, and some of its strains on Czapek-Dox's agar are dark green or bluish (Gams 1971). *Tolypocladium inflatum* and *T. cylindrosporum* not only have one type of conidia like *T. geodes*, but the conidiogenous cells of both species are ellipsoidal to subglobose at the lower part. In contrast, those of the cultured anamorph of *T. dujiaolongae* are cylindrical or only slightly swollen basally (Gams 1971). *Tolypocladium tundrense* resembles the anamorph of *T. dujiaolongae* in producing two types of conidia, but their conidial size and shape are different (Bissett 1983). Furthermore, these four species exhibit solitary and verticillate phialides (Table 1).

Gams (1971) established the genus *Tolypocladium* with the type species *T. inflatum*, and described three soil-inhabiting species, *T. inflatum*, *T. cylindrosporum* and *T. geodes*. Since then many species have been described in the genus. However, most *Tolypocladium* species have been described from soil samples and as a result their true role in the soil remains largely unknown. Although well known as saprobes commonly found in the soil, many *Tolypocladium* species are also entomopathogenic fungal parasites on a range of insects, rotifers, nematodes, and other fungi. Hodge & al. (1996) first established the relationship between *Tolypocladium* and *Cordyceps* sensu

TABLE 1. Morphological comparison of *Tolypocladium dujiaolongae* with allied *Cordyceps* and *Tolypocladium* species

SPECIES	CHARACTER				
	STIPE COLOUR	FERTILE PART	PERITHECIUM	ASCUS	ASCUS CAP (diam)
C. cicadae		Black brown	Perpendicularly immersed; 350–540 × 115–300 µm	262.5–378 × 6.2–9.1 µm	
T. paradoxum	Pale purple, whitish mycelial strands onto host	Dark purple	Wholly immersed; 480–500 × 300–350 µm	9–10 µm thick	4 µm
T. inegoense	White	Dark olive green	Superficial: 520–550 × 260–280 µm	400–450 × 7–7.5 µm	5–6.5 µm
C. imagamiana	White	Pallid ochre, oblong	Immersed 450–550 × 250–275 µm	7 µm thick	4 µm
T. dujiaolongae	Brown or yellow	Brown or black	Wholly immersed 600–780 × 270–360 µm	380–500 × 8–11 µm	5.5–6.5 µm

lato demonstrating that *Cordyceps subsessilis* Petch has a *Tolypocladium* anamorph.

Sung & al. (2007) recognised *Tolypocladium* and *Tolypocladium*-producing teleomorphs in the newly erected family *Ophiocordycipitaceae* (*Hypocreales*). Species were placed in the "C. ophioglossoides clade" and a new genus, *Elaphocordyceps* G.H. Sung & Spatafora, was erected to accommodate these taxa (Sung & al. 2007). However, following the 2011 Nomenclature Session of the XVIII International Botanical Congress in Melbourne, a single system of nomenclature, or "one fungus = one name" (1F = 1N), became effective 1 January 2013, regardless of life history states. In order to reflect changes in Article 59 of the ICN, Quandt & al. (2014) emended the family *Ophiocordycipitaceae* based on molecular phylogenetic analyses and proposed six genera encompassing *Drechmeria*, *Harposporium*, *Ophiocordyceps*, *Polycephalomyces, Purpureocillium*, and *Tolypocladium*; *Elaphocordyceps* is no longer a valid name in the 1F = 1N framework, and species were transferred to the genus *Tolypocladium*.

As with *I. cicadae* it would appear that *T. dujiaolongae* has probably also had a long history of usage in TCM under the general name of 'cicada flower'. We have found no documented records of human consumption of

T. dujiaolongae causing problems. Studies on the effects of artificial culture conditions on the artificial fruit body formation of *T. dujiaolongae*, submerged fermentation of strains, and detection of myriocin and other metabolites are ongoing by our team.

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Phylacia cylindrica sp. nov. from Brazil

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Abstract—A new species, *Phylacia cylindrica,* characterized by cylindrical stromata with constricted bases, is described from the southern region of the State of Bahia, Brazil. A key to *Phylacia* taxa found in Brazil is presented.

Key words—*Ascomycota*, Brazilian mycota, *Hypoxylaceae*, systematics

Introduction

The genus *Phylacia* Lév. was proposed by Léveillé (1845) with the type species *P. globosa* Lév. The main characteristics of the genus are carbonaceous stromata, a single layer of embedded, closely packed, elongated perithecia lacking ostioles, and a powdery spore mass (Dennis 1957). *Phylacia* is found mostly in the American tropics. Recently, this genus was transferred to *Hypoxylaceae* DC. (*Ascomycota*) as resurrected and emended by Wendt et al. (2018). Currently, nine taxa are accepted (Index Fungorum 2018), of which six have been recorded in Brazil (Pereira 2015, Speer 1980): *P. bomba* (Mont.) Pat. var. *bomba*, *P. globosa*, *P. poculiformis* (Mont.) Mont., *P. pseudohypoxylon* Speer, *P. surinamensis* (Berk. & M.A. Curtis) Dennis, and *P. turbinata* (Berk.) Dennis; the genus is represented in eleven Brazilian states (Amazonas, Bahia, Mato Grosso, Pará, Paraná, Pernambuco, Rio de Janeiro, Rio Grande do Sul, Rondônia, Roraima, and Santa Catarina). Although other genera of *Xylariaceae* in Brazil have been studied during the past decade (Pereira et al. 2008a,b, 2009, 2010; Trierveiler-Pereira et al. 2009; Yuyama et al. 2013), little research has

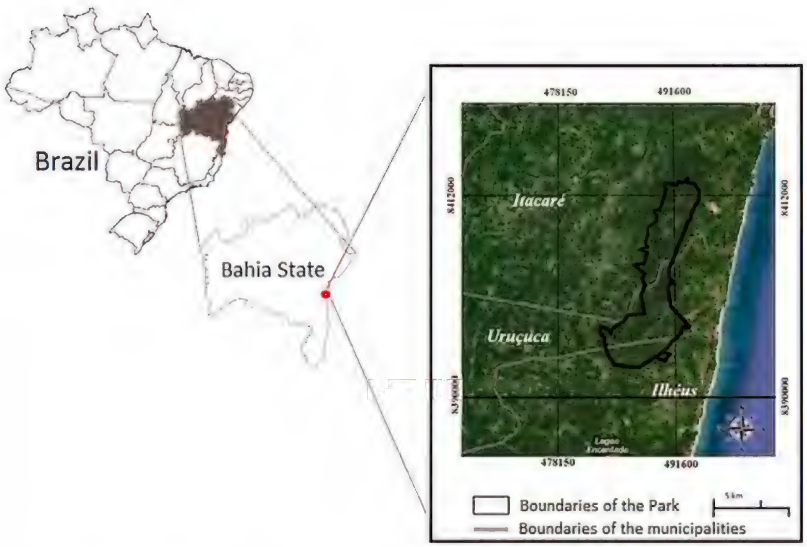

Fig 1. Geographical location of the Parque Estadual Serra do Conduru, Bahia, Brazil.

been conducted on *Phylacia* in Brazil, even though well represented in the tropics.

Below we describe a new *Phylacia* and provide a key to the seven taxa representing the genus in Brazil.

Materials & methods

In November 2012 a specimen of *Phylacia* was collected during a field trip to the Parque Estadual Serra do Conduru (PESC), a protected area of Atlantic Rainforest located in the municipalities of Ilhéus, Itacaré, and Uruçuca, State of Bahia, Brazil (Fig. 1). The specimen was analyzed according to Dennis (1957), Speer (1980), Rodrigues & Samuels (1989), and Medel et al. (2006). The holotype is deposited in the mycological collection of Herbário Centro de Pesquisas do Cacau, Ilhéus, Brazil (CEPEC) and the name registered in MycoBank. The isotype was deposited in the Tropical Fungarium (TFB) at the Universidade Estadual de Santa Cruz, Ilhéus, Brazil. A key to *Phylacia* taxa found in the Brazil is provided.

Taxonomy

Phylacia cylindrica L.T. Lacerda, J.L. Bezerra & Jad. Pereira, **sp. nov.** Figs 2–5

MycoBank MB805788

Differs from *Phylacia pseudohypoxylon* by its cylindrical stroma with constricted base and its smaller ascospores.

Type: Brazil. Bahia: Uruçuca, Parque Estadual Serra do Conduru, 14°20′S 39°02′W, on dead wood, 28-XI-2012, L.T. Lacerda & J. Pereira (CEPEC 2388, **holotype;** TFB 568, isotype).

Etymology: The epithet refers to the cylindrical form of the stroma.

Stromata solitary or gregarious, erumpent through bark, cylindrical, dark brown to black, 4–5 × 2–5 mm, surface smooth, carbonaceous, sessile or stipitate, base constricted, apex flat to slightly concave. KOH-extractable pigments purple vinaceous. Perithecia cylindrical, 2.5 × 0.4–0.5 mm, numerous. Asci not seen. Ascospores elliptical to sub-cylindrical, pale olive, 9.5–12.5 × 6–9.5 µm, smooth, without apparent germ slit. Perispore indehiscent in 10% KOH.

Figs 2–6. ***Phylacia cylindrica*** (holotype, CEPEC 2388). 2. Stromata. 3. Stromata showing constricted base. 4. Cross section showing inside of the stroma. 5. Sub-cylindrical ascospore without apparent germ slit. ***Phylacia surinamensis*** (holotype, K[M] 196019). 6. Ascospore with conspicuous germ slit. Bars: 2, 3 = 5 mm; 4 = 3 mm; 5, 6 = 5 µm.

Comments—Speer (1980) recorded *Phylacia pseudohypoxylon* from Ponta Grossa, Paraná State, Brazil, a species that differs from *P. cylindrica* by its subglobose sessile stromata (clavate to turbinate at maturity) and larger ascospores (14–15 × 4.5–5 µm).

We have examined the holotype of *P. surinamensis,* deposited in Kew Fungarium (K[M] 196019) and which differs from *P. cylindrica* in the following: a) smaller stromata (1.5–3 × 1–3 mm) that are sessile (but not stipitate) with a non-constricted base; b) KOH-extractable green pigments; c) longer and narrower ascospores (11–14 × 4–6 µm), with conspicuous germ slit seen in old ascospores (Fig. 6).

We were unfortunately unable to cultivate *P. cylindrica* on oatmeal agar, as had been done by Rodrigues & Samuels (1989) for *P. globosa, P. poculiformis,* and *P. bomba* var. *macrospora* K.F. Rodrigues & Samuels.

Key to *Phylacia* taxa recorded in Brazil

1a. Stromata sessile 2
1b. Stromata stipitate or pseudostipitate 4
2a. Stromata cylindrical, ascospores 11–14 × 4–6 µm ..... *P. surinamensis*
2b. Stromata hemispherical or subglobose 3
3a. Stromata hemispherical, ascospores 9–16.5 × 4–7.2 µm ..... *P. bomba* var. *bomba*
3b. Stromata subglobose, ascospores 14–15 × 4.5–5 µm ..... *P. pseudohypoxylon*
4a. Stromata pyriform, ascospores 10–15 × 5.5–7.5 µm ..... *P. poculiformis*
4b. Stromata not pyriform 5
5a. Stromata cylindrical with constricted base, ascospores 9.5–12.5 × 6–9.5 µm ..... *P. cylindrica*
5b. Stromata subglobose or turbinate 6
6a. Stromata subglobose, ascospores 11–15 × 7.5–9 µm ..... *P. globosa*
6b. Stromata turbinate, ascospores 11–13 × 5–6 µm ..... *P. turbinata*

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Lasiodiplodia cinnamomi sp. nov. from *Cinnamomum camphora* in China

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Abstract—A new species, *Lasiodiplodia cinnamomi*, is described as a pathogen causing branch canker of *Cinnamomum camphora* in Southern China. Morphology and phylogenetic analyses of ITS, RPB2, TEF1-α, and TUB2 sequence data support the position of the new species in *Lasiodiplodia*.

Key words —*Botryosphaeriaceae*, phylogeny, taxonomy

Introduction

Cinnamomum camphora is one of the principle tree species of China for ornamentation and timber (Li & al. 2007). In China, *Cinnamomum camphora* has been cultivated widely as street trees for its fast growth, fine wood, beautiful form, disease and insect resistance, resistance to pollution and acid rain (Wei 2008). During our investigation of plant diseases in Jiangsu Province, we found that several *Cinnamomum camphora* trees were infected by botryosphaeriaceous fungi, leading to tree death.

Fungi in *Botryosphaeriaceae* Theiss. & Syd. inhabit a wide diversity of hosts and substrates, including most economically and ecologically important trees and crops. *Lasiodiplodia* can be distinguished from related genera easily by the presence of pycnidial paraphyses and longitudinal striations on mature conidia (Phillips & al. 2013). In addition, the use of a combination of ITS and

TEF1-α sequences in the phylogenetic analysis of *Diplodia*, *Lasiodiplodia* and *Neofusicoccum* was suggested to separate species, because morphology is not a reliable character for species differentiation (Phillips & al. 2013).

Materials & methods

Samples and isolates

Fresh samples of *Lasiodiplodia* were collected from infected branches on *Cinnamomum camphora* in Jiangsu Province, China. Following a modified method of Fan & al. (2014), single conidia were isolated onto the surface of 1.8% PDA and incubated at 25 °C for up to 24 h. Single germinating conidia were removed onto fresh PDA plates. Two strains were used in this phylogenetic analysis. Specimens and isolates of the new species were deposited in the Museum of Beijing Forestry University, Beijing, China (BJFC). Axenic cultures are maintained in the China Forestry Culture Collection Center, Beijing, China (CFCC).

Morphological studies

Morphological observation was based on features of fruiting bodies produced on diseased plant tissues and cultural characteristics. Fruiting bodies were observed following the method of Slippers & al. (2013) and 50 spores were selected randomly for measurement using a Leica DM 2500 compound light microscope. Morphological or cultural characteristics, including colony color and texture, were observed on PDA after incubation at 25 °C, and recorded at 3 and 7 days.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from 7-day old colonies grown on PDA using a modified CTAB method (Doyle & Doyle 1990). DNA fragment size was checked with electrophoresis on 1% agarose gels, and the quality was measured by Thermo NanoDrop™ 2000 according to the user's manual (Desjardins & al. 2009). For the sequence data amplification of ITS, TEF1-α, TUB2, and RPB2, DNA Engine (PTC-200) Peltier Thermal Cycler was used with primers ITS5/ITS4 (White & al. 1990), EF1-688F/EF1-986R (Alves & al. 2008, Carbone & Kohn 1999), Bt2a/Bt2b (Glass & Donaldson 1995), and RPB2-LasF/RPB2-LasR (Cruywagen & al. 2017). The PCR amplification products were estimated visually by electrophoresis in 1.5% agarose gels. DNA sequencing was performed using an ABI PRISM® 3730XL DNA Analyzer with a BigDye® Terminator Kit v.3.1 at the Shanghai Invitrogen Biological Technology Company Limited (Beijing, China).

DNA sequence analysis

Sequences from this study and reference sequences obtained from GenBank (Table 1) were aligned using MAFFT v.6 (Katoh & Toh 2010) and edited manually using MEGA6 (Tamura & al. 2013). Phylogenetic analyses were generated by PAUP v.4.0b10 for maximum parsimony (MP) analysis (Swofford 2003), PhyML v.7.2.8 for maximum likelihood (ML) analysis (Guindon & al. 2010), and MrBayes v.3.1.2 for

Table 1. Sequences of *Lasiodiplodia* spp. and *Diplodia mutila* outgroup used in phylogenetic analyses

Species	Strain	Host	Origin	GenBank Number			
				ITS	EF1-α	TUB	RPB2
Diplodia mutila	CMW 7060	*Fraxinus*	Netherlands	AY236955	AY236904	AY236933	EU339574
L. avicenniae	CMW41467*	*Avicennia*	South Africa	KP860835	KP860680	KP860758	KU587878
	LAS199	*Avicennia*	South Africa	KU587957	KU587947	KU587868	KU587880
L. brasiliensis	CMM 4015*	*Mangifera*	Brazil	JX464063	JX464049	—	—
	CMM 4469	*Anacardium*	Brazil	KT325574	KT325580	—	—
L. bruguierae	CMW41470*	*Bruguiera*	South Africa	KP860833	KP860678	KP860756	KU587875
	CMW 42480	*Bruguiera*	South Africa	KP860832	KP860677	KP860755	KU587876
L. caatinguensis	CMM 1325*	*Citrus*	Brazil	KT154760	KT008006	KT154767	—
	IBL 381	*Spondias*	Brazil	KT154757	KT154751	KT154764	—
L. chinensis	CGMCC 3.18061*	Unknown	China	KX499889	KX499927	KX500002	KX499965
	CGMCC 3.18044	*Vaccinium*	China	KX499875	KX499913	KX499988	KX499951
L. cinnamomi	**CFCC 51997***	***Cinnamomum***	**China**	MG866028	MH236799	MH236797	MH236801
	CFCC 51998	***Cinnamomum***	**China**	MG866029	MH236800	MH236798	MH236802
L. citricola	CBS 124707*	*Citrus*	Iran	GU945354	GU945340	KU887505	KU696351
	CBS 124706	*Citrus*	Iran	GU945353	GU945339	KU887504	KU696350
L. crassispora	CMW 13448	*Eucalyptus*	Venezuela	DQ103552	DQ103559	KU887504	KU696350
	CBS 118741*	*Santalum*	Australia	DQ103550	DQ103557	KU887506	KU696353
L. euphorbiaceicola	CMW 33350	*Adansonia*	Botswana	KU887149	KU887026	KU887455	KU696346
	CMW 36231	*Adansonia*	Zimbabwe	KU887187	KU887063	KU887494	KU696347
L. exigua	CBS 137785*	*Retama*	Tunisia	KJ638317	KJ638336	KU887509	KU696355
	BL 184	*Retama*	Tunisia	KJ638318	KJ638337	—	—
L. gilanensis	CBS 124704*	—	Iran	GU945351	GU945342	KU887511	KU696357
	CBS 124705	—	Iran	GU945352	GU945341	KU887510	KU696356

L. gonubiensis	CMW 14077*	*Syzygium*	South Africa	AY639595	DQ103566	DQ458860	KU696359
	CMW 14078	*Syzygium*	South Africa	AY639594	DQ103567	EU673126	KU696358
L. gravistriata	CMM 4564*	*Anacardium*	Brazil	KT250949	KT250950	—	—
	CMM 4565	*Anacardium*	Brazil	KT250947	KT266812	—	—
L. hormozganensis	CBS 124709*	*Olea*	Iran	GU945355	GU945343	KU887515	KU696361
	CBS 124708	*Mangifera*	Iran	GU945356	GU945344	KU887514	KU696360
L. hyalina	CGMCC 3.17975	*Acacia*	China	KX499879	KX499917	KX499992	KX499955
	CGMCC 3.18383	Tree	China	KY767661	KY751302	KY751299	KY751296
L. iraniensis	IRAN 1520C	*Salvadora*	Iran	GU945348	GU945336	KU887516	KU696363
	IRAN 1502C	*Juglans*	Iran	GU945347	GU945335	KU887517	KU696362
L. laeliocattleyae	BOT-10*	*Mangifera*	Egypt	JN814397	JN814424	KU887508	KU696354
	BOT-29	*Mangifera*	Egypt	JN814401	JN814428	—	—
L. lignicola	CBS 134112*	Wood	Thailand	JX646797	KU887003	JX646845	KU696364
	MFLUCC 11-0656	Wood	Thailand	JX646798	—	JX646846	—
L. macrospora	CMM 3833*	*Jatropha*	Brazil	KF234557	KF226718	KF254941	—
L. mahajangana	CMW 27801*	*Terminalia*	Madagascar	FJ900595	FJ900641	FJ900630	KU696365
	CMW 27818	*Terminalia*	Madagascar	FJ900596	FJ900642	FJ900631	KU696366
L. margaritacea	CBS 122519*	*Adansonia*	Australia	EU144050	EU144065	KU887520	KU696367
	CBS 122065	*Adansonia*	Australia	EU144051	EU144066	—	—
L. mediterranea	CBS 137783*	*Quercus*	Italy	KJ638312	KJ638331	KU887521	KU696368
	CBS 137784	*Vitis*	Italy	KJ638311	KJ638330	KU887522	KU696369
L. missouriana	CBS 128311*	*Vitis*	USA	HQ288225	HQ288267	HQ288304	KU696370
	CBS 128312	*Vitis*	USA	HQ288226	HQ288268	HQ288305	KU696371
L. parva	CBS 456.78*	*Manihot* soil	Colombia	EF622083	EF622063	KU887523	KU696372
	CBS 495.78	*Manihot* soil	Colombia	EF622084	EF622064	EU673114	KU696373

L. plurivora	STE-U 5803*	*Prunus*	South Africa	EF445362	EF445395	KU887524	KU696374
	STE-U 4583	*Vitis*	South Africa	AY343482	EF445396	KU887525	KU696375
L. pontae	CMM1277	*Spondias*	Brazil	KT151794	KT151791	KT151797	—
L. pseudotheobromae	CBS 116459*	*Gmelina*	Costa Rica	EF622077	EF622057	EU673111	KU696376
	CGMCC 3.18047	*Pteridium*	China	KX499876	KX499914	KX499989	KX499952
L. pyriformis	CBS 121770*	*Acacia*	Namibia	EU101307	EU101352	KU887527	KU696378
	CBS 121771	*Acacia*	Namibia	EU101308	EU101353	KU887528	KU696379
L. rubropurpurea	WAC 12535*	*Eucalyptus*	Australia	DQ103553	DQ103571	EU673136	KU696380
	WAC 12536	*Eucalyptus*	Australia	DQ103554	DQ103572	KU887530	KU696381
L. sterculiae	CBS 342.78*	*Sterculia*	Germany	KX464140	KX464634	KX464908	KX463989
L. subglobosa	CMM 3872*	*Jatropha*	Brazil	KF234558	KF226721	KF254942	—
	CMM 4046	*Jatropha*	Brazil	KF234560	KF226723	KF254944	—
L. thailandica	CGMCC 3.18382	*Podocarpus*	China	KY767662	KY751303	KY751300	KY751297
	CGMCC 3.18384	*Albizia*	China	KY767663	KY751304	KY751301	KY751298
L. theobromae	CBS 164.96*	Fruit	New Guinea	AY640225	AY640258	KU887532	KU696383
	CBS 111530	—	—	EF622074	EF622054	KU887531	KU696382
L. venezuelensis	WAC 12539*	*Acacia*	Venezuela	DQ103547	DQ103568	KU887533	KU696384
	WAC 12540	*Acacia*	Venezuela	DQ103548	DQ103569	KU887534	—
L. viticola	CBS 128313*	*Vitis*	USA	HQ288227	HQ288269	HQ288306	KU696385
	UCD 2604MO	*Vitis*	USA	HQ288228	HQ288270	HQ288307	KU696386
L. vitis	CBS 124060*	*Vitis*	Italy	KX464148	KX464642	KX464917	KX463994

New sequences are set in bold font. Ex-type/ex-epitype isolates are marked by *.

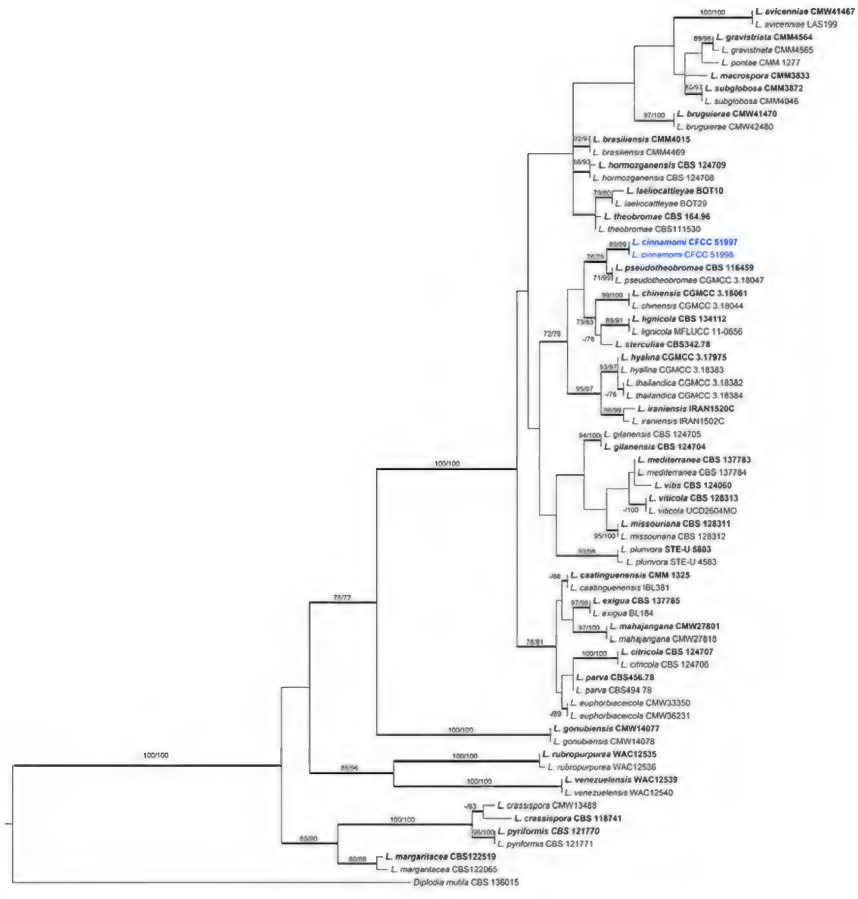

Plate 1. Phylogram of ITS, RPB2, TEF1-α, and TUB2 regions based on MP analysis. (The ML and BI analyses produced congruent trees.) Values above the branches indicate maximum parsimony bootstrap (MP BP ≥70%) and maximum likelihood bootstrap (ML BP ≥70 %). Thickened branches represent posterior probabilities ≥0.90 from BI. Scale bar = 20 nucleotide substitutions. Newly generated sequences are set in blue font. Ex-type strains are in bold font.

Bayesian Inference (BI) (Ronquist & Huelsenbeck 2003). The phylogenetic tree was constructed using the combined multi-gene dataset to compare our new *Lasiodiplodia* sequences with other ex-type material sequenced in recent studies (Alves & al. 2007, Phillips & al. 2013, Slippers & al. 2013, Linaldeddu & al. 2015).

We selected *Diplodia mutila* (Fr.) Mont. (CBS 136015) as outgroup (Phillips & al. 2013). Trees are shown using FigTree v.1.3.1 (Rambaut & Drummond 2010). MP analysis was performed by a heuristic search option of 1000 random-addition sequences with a tree bisection and reconnection (TBR) algorithm. Maxtrees were set to 5000, branches of zero length were collapsed and all equally parsimonious trees were saved. Other calculated parsimony scores were tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency (RC). ML analysis was also performed with a GTR site substitution model (Guindon & al. 2010). The branch support was evaluated with a bootstrapping (BS) method of 1000 replicates (Hillis & Bull 1993). For the BI analysis, a Markov Chain Monte Carlo (MCMC) algorithm was performed (Rannala & Yang 1996). The models of evolution were estimated by MrModeltest v.2.3 (Posada & Crandall 1998). Sequence data were deposited in GenBank (Table 1). The multilocus sequences alignment file was deposited in TreeBASE (www.treebase.org) as accession S21480. The taxonomic novelty was deposited in MycoBank (Crous & al. 2004).

Results

Molecular phylogeny

The combined gene (ITS, TEF1-α, TUB2, and RPB2) data matrix contains 68 strains from 36 *Lasiodiplodia* species, and one outgroup strain. The statistics for the parsimony analysis revealed that from the remaining 1611 characters, 1300 characters were constant, 82 variable characters were parsimony-uninformative and 229 characters were parsimony informative. The MP analysis yielded one most parsimonious tree (TL = 529, CI = 0.688, RI = 0.858, RC = 0.591), shown in Plate 1. The phylogenetic tree obtained from ML and Bayesian analyses with the MCMC algorithm was consistent with the illustrated MP tree. Separate bootstrap values >50% for the MP and the ML analyses are presented above the nodes (as "MP/ML"); and BI values ≥0.90 are indicated by thickened branches.

Taxonomy

Lasiodiplodia cinnamomi C.M. Tian & Ning Jiang, **sp. nov.** Plate 2

MycoBank MB 822574

Differs from *Lasiodiplodia pseudotheobromae* by its smaller conidia.

Type: China, Jiangsu Prov., Gaoyou City, 32°47′25″N 119°28′12″E, 1 m asl, on twigs and branches of *Cinnamomum camphora* (L.) J. Presl (*Lauraceae*), 12 February 2017, coll. N. Jiang (**Holotype**, BJFC-S1374; ex-type culture CFCC 51997; GenBank ITS MG866028, EF1-α MH236799, TUB MH236797, RPB2 MH236801).

Etymology: *cinnamomi* (Lat.), named after the host genus.

Conidiomata formed on hosts, pycnidial, multilocular, dark brown to black, semi-immersed in the host becoming erumpent when mature. Paraphyses

Plate 2. Morphology of *Lasiodiplodia cinnamomi* from *Cinnamomum camphora* (BJFC-S1374). A: Habit of conidiomata on stem; B: Transverse sections through conidiomata; C: Longitudinal sections through conidiomata; D: Colonies on PDA at 3 days; E: Colonies on PDA at 10 days; F. Transverse sections through conidiomata; G–I: Conidiogenous layer with conidia developing on conidiogenous cells between paraphyses; J: Conidiogenous cells; K, L: Immature conidia; M, N: Mature conidia in two different focal planes to show the longitudinal striations. Scale bars: B, C, F = 100 μm, G–N = 10 μm.

hyaline, cylindrical, aseptate, sometimes branched, ends rounded, ≤106 × 3–4 μm, arising amongst the conidiogenous cells. Conidiogenous cells hyaline, smooth, cylindrical, slightly swollen at the base, holoblastic, proliferating percurrently to form one or two closely spaced annellations, 10.4–13.6 × 2.4–6.3 μm. Conidia ellipsoidal, apex and base rounded, widest at the middle, thick-walled, initially hyaline and aseptate and remaining so for a long time,

becoming 1-septate and dark brown only some time after release from the conidiomata, with melanin deposits on the inner surface of the wall arranged longitudinally giving a striate appearance to the conidia, (17.5–)18.7–21.1 (–22.4) × (11.5–)12.7–14.1(–15.5) µm, 95% confidence limits = 18.7–21.1 × 12.7–14.1 µm (x ± S.D. = 19.9 ± 1.2 × 13.4 ± 0.7 µm, l/w ratio = 1.5 ± 0.1). Colonies initially white to light-brown with fluffy, aerial mycelium, becoming grey on the surface after 3 days; reverse side of the colonies dark-brown. Sexual morph not observed.

Host/Distribution: on branches of *Cinnamomum camphora* in China.

Additional specimen examined: **CHINA, Jiangsu Prov., Gaoyou City**, 32°47′25″N 119°28′12″E, 1 m asl, on twigs and branches of *Cinnamomum camphora,* 12 February 2017, coll. N. Jiang (BJFC-S1375; ex-type culture CFCC 51998; GenBank ITS MG866029, EF1-α MH236800, TUB MH236798, RPB2 MH236802).

Note: Two strains representing *L. cinnamomi* clustered in a supported clade and this species is considered to be a distinct species based on molecular data. The most closely related species in the phylogram is *L. pseudotheobromae* A.J.L. Phillips & al., which differs morphologically by its larger conidia (22.5–33 × 13.5–20 µm; Alves & al. 2008).

Discussion

In this study, two strains of *Lasiodiplodia* associated with camphor canker disease in China were identified. Species identification was supported by morphology and multigene DNA data (ITS, TEF1-α, TUB, and RPB2), which indicated that *Lasiodiplodia cinnamomi* represents a distinct species (Plate 1). Although *L. cinnamomi* represents a sister group to *L. pseudotheobromae*, the two species can be distinguished by conidial size. Hence, we regard this taxon as a novel species of *Lasiodiplodia*. It should be noted that characteristic mature conidia of *L. cinnamomi* are not easily observed from conidiomata on bark, because the conidia have already been released when mature.

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The *Heterobasidion insulare* complex from Pakistan

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Abstract—*Heterobasidion linzhiense* and *H. orientale* were collected from *Pinus wallichiana* forests. Both species are described and illustrated based on the western Himalayan specimens. Combined nrDNA ITS and LSU sequence analysis supports the identity of these species in *Heterobasidion insulare* complex. They represent new species record from pristine forested areas of Pakistan, and *Pinus wallichiana* is recorded as new host for both species.

Key words—*Bondarzewiaceae*, macrofungi, phylogeny, polypore, *Russulales*

Introduction

Heterobasidion Bref. (*Bondarzewiaceae*, *Russulales*) is a well-known polypore genus characterized by resupinate to pileate basidiocarps, a dimitic hyphal system with mostly simple septate generative hyphae and dextrinoid skeletal hyphae, and finely asperulate inamyloid and nondextrinoid basidiospores (Gilbertson & Ryvarden 1987). The genus is indigenous to many areas and an important ecological factor involved in nutrient cycling, forest regeneration, and forest succession (Garbelotto 2004, Chen & al. 2015). *Heterobasidion* has a worldwide negative impact on conifers, both ecologically and economically, by reducing site productivity and the amount of harvestable timber (Woodward & al. 1998). About twelve species have been accepted in *Heterobasidion* (Buchanan 1988, Niemelä & Korhonen 1998, Ota & al. 2006, Dai & al. 2007, Dai & Korhonen 2009, Tokuda & al. 2009, Otrosina & Garbelotto 2010, Chen

& al. 2014). Five species are currently recognized in the *H. annosum* complex: *H. abietinum* Niemelä & Korhonen, *H. annosum* (Fr.) Bref., *H. irregulare* Garbel. & Otrosina, *H. occidentale* Otrosina & Garbel., and *H. parviporum* Niemelä & Korhonen (Niemelä & Korhonen 1998; Otrosina & Garbelotto 2010). Seven species are recognized in the *H. insulare* complex: *H. amyloideum* Y.C. Dai & al., *H. australe* Y.C. Dai & Korhonen, *H. ecrustosum* Tokuda & al., *H. insulare* (Murrill) Ryvarden, *H. linzhiense*, *H. orientale,* and *H. tibeticum* Y.C. Dai & al. (Ota & al. 2006, Dai & al. 2007, Dai & Korhonen 2009, Tokuda & al. 2009, Chen & al. 2014). Most species in the *H. insulare* complex are nonpathogenic and have larger basidiospores than species in the *H. annosum* complex. Another species of *Heterobasidion* outside of these two complexes, *H. araucariae* P.K. Buchanan, occurs in Australia, New Zealand, and adjacent regions (Buchanan 1988). Previously, only *H. insulare* has been reported from Pakistan (Ahmad & al. 1997). During our present exploration of macrofungi, *H. linzhiense* and *H. orientale* were collected from coniferous forests of western Himalayas. Both morphological and molecular evidence were used to confirm the identity of these species.

Materials & methods

Morphological evaluation

Basidiomata were collected, photographed, dried, characterized morphologically, and vouchered in the Herbarium, Department of Botany, University of the Punjab, Lahore, Pakistan (LAH) and the Farlow Herbarium, Harvard University, Cambridge MA, USA (FH). Microscopic observations were made from slide preparations of dried specimens stained with Cotton Blue and Melzer's reagent under a Meiji MX4300H biological microscope. Anatomical features (basidiospores, basidia, cystidia, hyphae) were measured from at least 25 elements under oil immersion at 1000×; x = arithmetic mean of spore length and spore width for all spores measured. Line drawings were made with a camera lucida.

DNA extraction, PCR amplification, DNA sequencing

Genomic DNA was extracted from small pieces of basidiomata by a modified CTAB method (Bruns 1995) and using the Qiagen DNeasy Plant Mini Kit (cat. no. 69104). The internal transcribed spacer region (ITS1/5.8S/ITS2) was targeted with primer pair ITS1F/ITS4 and the large subunit (LSU) with primer pair LROR/LR5 (White & al. 1990, Gardes & Bruns 1993). PCR was carried out using Econo Taq DNA Polymerase under the following cycling parameters: initial denaturation (94 °C for 1 min), 35 elongation cycles (94 °C for 1 min, 53 °C for 1 min, and 72 °C for 1 min), and final extension 72 °C (8 min). Amplified PCR products were purified and sequenced bidirectionally by Macrogen (Republic of Korea).

Table 1. Sequences of *Heterobasidion* spp. (and *Bondarzewia* outgroup) used in phylogenetic analysis. New sequences are set in bold font.

Taxon	Sample	Origin	Host	GenBank	
				ITS	nLSU
H. abietinum	00057/2	Italy	*Abies alba*	KJ651453	KJ651511
	00051/1	Italy	*Picea abies*	KJ651450	KJ651508
H. amyloideum	Li 1878	China	Unknown	KJ651455	KJ651513
	Li 1675	China	Unknown	KJ651454	KJ651512
H. annosum	09001/1	Italy	*Pinus nigra*	KJ651461	KJ651519
	K 06129/6	Russia	*Pinus sylvestris*	KJ583211	KJ583225
H. australe	K 05172/3	China	*Tsuga chinensis*	KJ651466	KJ651524
	K 05175/2	China	*Tsuga chinensis*	KJ651467	KJ651525
H. ecrustosum	K 07103/2	China	*Pinus* sp.	KJ651471	KJ651529
	K 08145/2	China	Unknown	KJ651472	KJ651530
H. linzhiense	***MSM#0097***	***Pakistan***	***Pinus wallichiana***	***MH233930***	***MH233931***
	Cui 9707	China	*Pinus* sp.	KJ651483	KJ651541
	Dai 5408	China	*Picea* sp.	KJ651484	KJ651542
H. parviporum	08129/5	Russia	*Picea abies*	KJ651501	KJ651559
	04121/3	Finland	*Picea abies*	KJ583212	KJ583226
H. orientale	**MSM#0099**	**Pakistan**	***Pinus wallichiana***	**MH233932**	**MH233933**
	K 00085/2	China	*Abies* sp.	KJ651492	KJ651550
	K 00082/6	China	Unknown	KJ651491	KJ651549
H. tibeticum	Dai 5537	China	*Pinus* sp.	KJ651507	KJ651565
	Dai 5534	China	*Pinus* sp.	KJ651506	KJ651564
B. montana	AFTOL-ID 452	—	—	DQ234539	DQ200923

Sequence alignment and phylogenetic analysis

Sequencing of the two *Heterobasidion* specimens (MSM#0098; MSM#0099) yielded fragments of 700 base pairs for ITS and 1160 base pairs for LSU. Attempted amplification of the RPB1 and RPB2 genes was unsuccessful for both taxa. Closely related ITS and LSU sequences were retrieved from GenBank based on initial BLAST results and following Chen & al. (2015). Information on all the retrieved sequences used to construct phylogenetic tree is provided in Table 1.

Sequences were manually edited and assembled using BioEdit (www.mbio.ncsu.edu/bioedit/bioedit.html). Following Dentinger & al. (2011) for complete ITS sequences, all sequences were trimmed with the conserved motifs

5′–(...GAT)CATTA– and –GACCT(CAAA...)–3′ with the intervening alignment portion included in analysis. *Bondarzewia montana* (Pers.) Harmaja (DQ200923) was used as outgroup based on results reported by Chen & al. (2015).

Our new sequences were aligned with GenBank sequences from related taxa using ClustalX (Thompson & al. 1997) and manually edited using BioEdit (Hall 1999). Maximum Likelihood analysis was conducted using RAxML 7.2.8 (Stamatakis 2006) based on the GTR + G nucleotide substitution model. The topology was assessed by 1000 bootstrap replicates.

Taxonomy

Heterobasidion linzhiense Y.C. Dai & Korhonen,
Ann. Bot. Fenn. 44(2): 141 (2007). FIG. 1

BASIDIOCARP annual, pileate, sessile, solitary, leathery when fresh, corky when dry. PILEUS semicircular, 4–6 cm wide, and 7.5 mm thick at base, cream buff, reddish brown at one edge, azonate; margin sharp, undulating. PORE SURFACE cream to buff-yellow when dry; pores angular, 3–4 per mm. TUBES cream buff to buff-yellow, corky, ≤5 mm long. ODOR AND TASTE not recorded.

HYPHAL SYSTEM dimitic: generative hyphae without clamp connections, hyaline, occasionally branched, simple septate, thick-walled, 3–5.5 µm diam.; skeletal hyphae dominant, hyaline, rarely branched, interwoven, thick-walled with a lumen, 3.6–6 µm diam. TUBES: Generative hyphae hyaline, thin-walled, frequently simple septate, rarely branched, parallel along the tubes, 2–4.5 µm diam.; skeletal hyphae dominant, hyaline, thick-walled, occasionally branched, interwoven, 2.8–5.5 µm diam. CYSTIDIA absent. CYSTIDIOLES present, thin-walled, hyaline, 14–24.8 × 3.2–5.8 µm; basidia clavate, four-spored, 14–19 × 5–7 µm. BASIDIOLES similar to basidia, but smaller in size. BASIDIOSPORES broadly ellipsoid to subglobose, hyaline, finely asperulate, usually guttulate, 6–7.3 × 5.6–7 µm [x = 6.8 × 6 µm].

MATERIAL STUDIED: **PAKISTAN**, **KHYBER PAKHTUNKHWA**, **Swat**, Mankial, on *Pinus wallichiana* A.B. Jacks., 5 September 2013, Malka Saba & Abdul Nasir Khalid, MSM#0097 (FH00304577, LAH310093; GenBank MH233930, MH233931).

COMMENTS—Our Pakistani specimen is morphologically similar to the protologue description of *H. linzhiense* (Dai & al. 2007). *Heterobasidion linzhiense,* originally described from Linzhi County, Xizang (Tibet), China, is separated from the other *Heterobasidion* species by smaller pores on the undersurface of basidiomata, larger basidiospores, and subulate cystidioles (Dai & al. 2007).

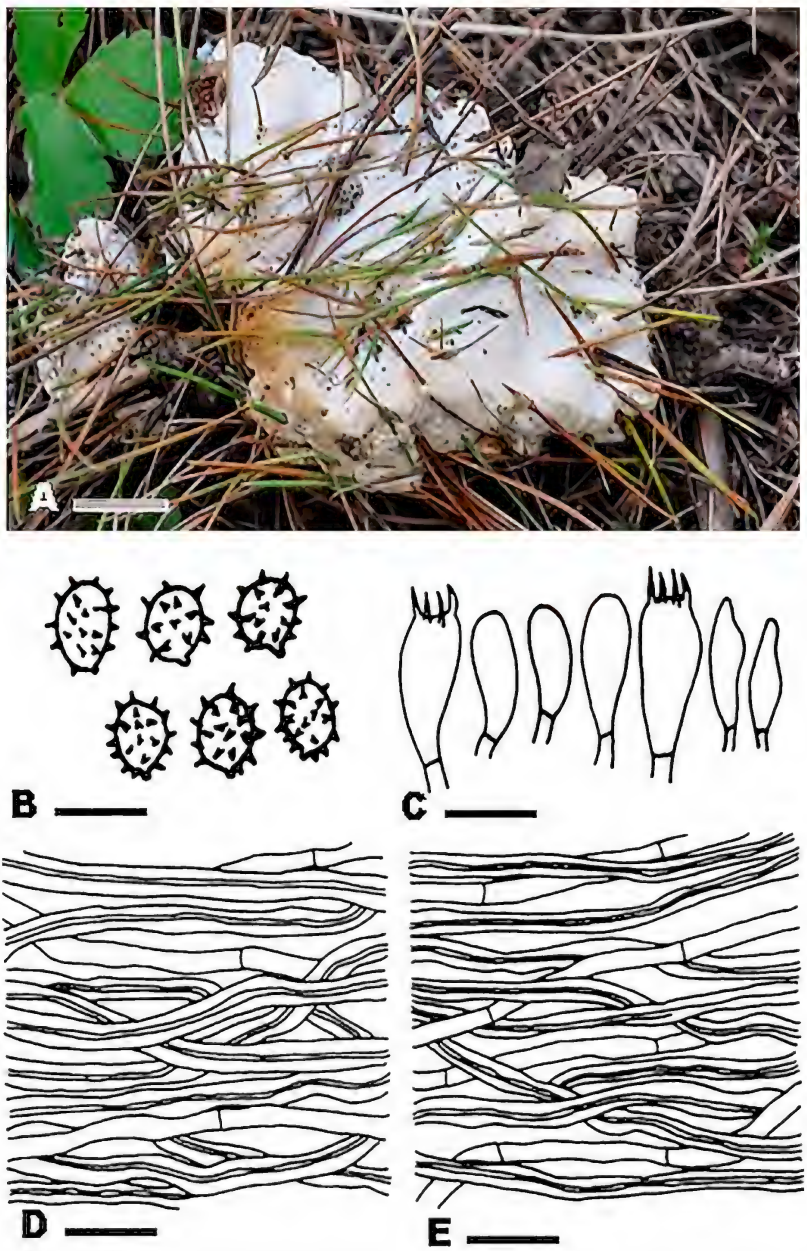

FIG. 1. *Heterobasidion linzhiense* (MSM#0097): A, Basidiomata; B, Basidiospores; C, Basidia; basidioles, and cystidioles; D, Hyphae from trama; E, Hyphae from context. Scale bars: A = 10 mm; B = 5 μm; C–E = 10 μm.

Heterobasidion orientale Tokuda, T. Hatt. & Y.C. Dai, Mycoscience 50(3): 193 (2009). FIG. 2

BASIDIOCARP annual, pileate, sessile, solitary, lacerate, leathery when fresh, corky when dry. PILEUS semicircular, 13.5 × 2 cm, zonate, reddish brown, cream on the edge; margin sharp or blunt, undulating. PORE surface cream to buff-yellow when dry; pores round, angular or labyrinthiform, 1–4 per mm. TUBES cream buff to buff-yellow, corky, ≤8 mm long. ODOR AND TASTE not recorded.

HYPHAL SYSTEM dimitic: generative hyphae without clamp connections, hyaline, occasionally branched, simple septate, thick-walled; skeletal hyphae dominant, hyaline, rarely branched, interwoven, thick-walled with a lumen, 3–6.5 µm diam. TUBES: Generative hyphae hyaline, thin-walled, frequently simple septate, rarely branched, parallel along the tubes, 2–4.5 µm diam.; skeletal hyphae dominant, hyaline, thick-walled, occasionally branched, interwoven, 3–6 µm diam.. CYSTIDIA absent. BASIDIA clavate, four spored, 14–26 × 7–10 µm. BASIDIOLES similar to basidia, but smaller in size. BASIDIOSPORES globose to subglobose, hyaline, finely asperulate, 4.5–6 × 4–5 µm, [x = 5.5 × 4.3 µm].

MATERIAL STUDIED: **PAKISTAN**, **KHYBER PAKHTUNKHWA**, **Shangla**, Yakh Tangay, on *Pinus wallichiana*, 2 September 2013, Malka Saba & Abdul Nasir Khalid, MSM#0099 (FH00304569, LAH310093; GenBank MH233932, MH233933).

COMMENTS—Our Pakistani specimen is morphologically similar to the protologue description of *H. orientale* (Jang & al. 2007). *Heterobasidion orientale* is characterized by sessile to effused-reflexed annual basidiocarps covered by a thin crust, a reddish brown pileus with a marginal white zone, and larger regular to labyrinthiform pores. This species shared genetic similarity with *H. australe* and *H. tibeticum* in nrDNA sequences (FIG. 3). Morphologically, presence of large pores on the undersurface (1–4/mm), the absence of a glazed pore surface, and slightly larger spores distinguish *H. orientale* from *H. australe*, which is also excluded by the annual habit, larger pores, and absence of amyloid context hyphae (Chen & al. 2014) characterizing our Pakistani material.

Phylogeny

Our final dataset comprises twenty ingroup and one outgroup sequences, two of which were generated during this study. After removing and editing the ambiguous letters from the aligned sequences, we phylogenetically compared 1935 positions of which 1778 characters were conserved, 148 were variable, 41 were parsimony informative, and 107 were singletons.

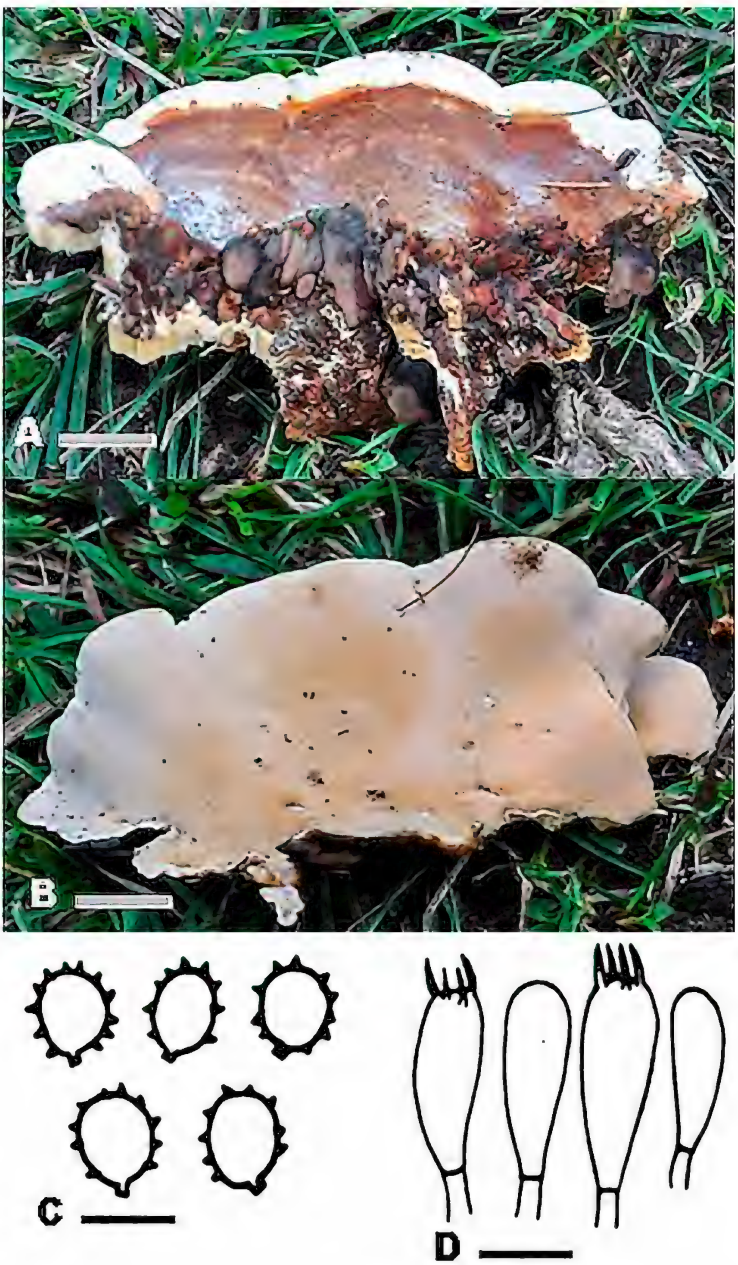

Fig. 2. *Heterobasidion orientale* (MSM#0099): A, B, Basidiomata; C, Basidiospores; D, Basidia and basidioles. Scale bars: A, B = 12 mm; C = 5 µm; D = 10 µm.

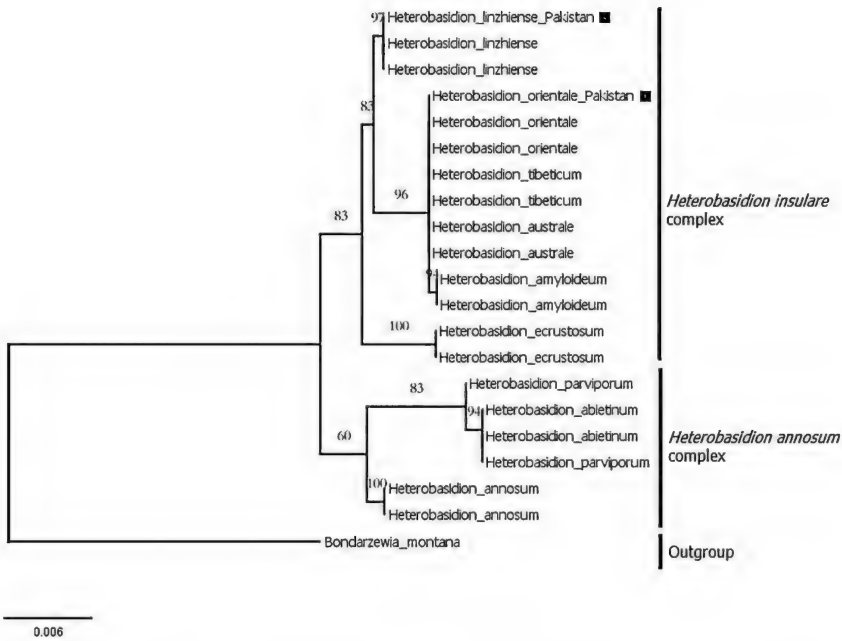

FIG. 3. Phylogenetic relationship of Pakistani *Heterobasidion* spp. and allied species based on combined dataset of nrDNA ITS + LSU sequences using maximum likelihood (ML) method. Bootstrap values >50% are cited above the nodes. Sequences generated during this study are labeled with ■.

Phylogenetic trees inferred using ITS sequences and ITS+LSU sequences showed no topological differences; we display only the combined ITS+LSU-based tree (FIG. 3). Species in the tree are divided into two *Heterobasidion* groups, the *H. insulare* complex and *H. annosum* complex, with the two new Pakistani sequences clustered in the *H. insulare* complex.

The Pakistani *H. linzhiense* sequence clustered with the other *H. linzhiense* sequences with strong (97%) bootstrap support.

The Pakistani *H. orientale* sequence clustered with sequences of *H. australe*, *H. orientale*, and *H. tibeticum* due to high genetic similarity in their rDNA sequences; we identified the Pakistani specimen based on its close morphological similarities with *H. orientale*. There are no differences in the ITS and LSU sequences among the three species. *Heterobasidion orientale* has nomenclatural priority and would be the correct name if the three species are found to be synonymous.

Acknowledgements

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Neopestalotiopsis rosicola sp. nov. causing stem canker of *Rosa chinensis* in China

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Abstract—Two specimens of *Neopestalotiopsis* were collected from China rose in Jiangsu Province, China. Cankers and conidiomata were formed on stems of the infected hosts. Phylogenetic analysis of the combined ITS, tef1, and tub2 DNA markers revealed that both strains were conspecific and different from all other described *Neopestalotiopsis* species. The two strains are described and illustrated here as a new species, *Neopestalotiopsis rosicola*, and compared with related species genetically, morphologically and in host association. This is the first report of *Neopestalotiopsis* species causing China rose stem canker in China.

Key words —morphology, *Sporocadaceae,* phylogeny, taxonomy

Introduction

Roses represent one of the most important groups of horticultural and ornamental plants throughout the world (Krüssmann 1981). China rose (*Rosa chinensis*) is cultivated widely in cities as an ornamental flower and used in traditional Chinese medicine (Han & al. 2017). Disease management in large scale rose-producing plantations is generally effective in preventing plant infection; however, during our investigation we discovered roses killed by fungal pathogens.

Species of *Neopestalotiopsis* (*Sporocadaceae;* Jaklitsch & al. 2016, Hongsanan & al. 2017), many previously treated as *Pestalotiopsis,* include important plant pathogens (Maharachchikumbura & al. 2013, Jayawardena

& al. 2015) and plant endophytes (Watanabe & al. 2010, Debbab & al. 2013). Maharachchikumbura & al. (2014) split *Pestalotiopsis* sensu lato into three genera—*Neopestalotiopsis*, *Pseudopestalotiopsis*, *Pestalotiopsis* sensu stricto—based on morphology and phylogeny. *Neopestalotiopsis* with the generic type *N. protearum* (Crous & L. Swart) Maharachch. & al. could be distinguished morphologically from *Pseudopestalotiopsis* and *Pestalotiopsis* sensu stricto by its versicolorous median cells and conidiophores often reduced to conidiogenous cells (Maharachchikumbura & al. 2014) and molecularly by *Neopestalotiopsis* forming a distinct clade in the LSU phylogeny (Maharachchikumbura & al. 2014). Various *Neopestalotiopsis* species are known to cause diseases, e.g., *N. vitis* Jayaward. & al. causing a leaf spot disease of grapevine (Jayawardena & al. 2016). Based on morphological and phylogenetic analyses of ITS, TEF1, and TUB2, we describe a new *Neopestalotiopsis* species causing a disease of *Rosa chinensis* in southern China.

Materials & methods

Samples and isolates

Fresh *Neopestalotiopsis* specimens were collected from infected stems or twigs on *Rosa chinensis* in Jiangsu Province, China. Single conidial isolates were obtained from fruiting bodies by removing a mucoid conidial mass from pycnidial ostioles following a modified method of Fan & al. (2014), spreading the suspension on the surface of 1.8% potato dextrose agar (PDA), and incubating at 25 °C for up to 24 h. Single germinating conidia were removed and plated onto fresh potato dextrose agar (PDA). Two strains were used in the phylogenetic analysis (TABLE 1). Specimens and isolates of the new species are deposited in the Museum of Beijing Forestry University, Beijing, China (BJFC). Axenic cultures are maintained in the China Forestry Culture Collection Center, Beijing, China (CFCC).

Morphological studies

Fruiting bodies were observed morphologically on diseased plant tissues and in culture following Maharachchikumbura & al. (2012). Fifty spores were selected randomly for measurement using a Leica DM 2500compound light microscope. Colony characteristics observed and recorded from cultures incubated on PDA in the dark at 25°C at 3, 7, and 15 days included colony color, texture, and the arrangement of the conidiomata.

Pathogenicity test

Healthy China rose stems (*Rosa chinensis*) were surface sterilized according to Feng & al. (2014). Considering China roses are planted in cities and are pruned, we applied a wound on stems of the treatment and control groups and covered the wound either with mycelium or with pure PDA. The inoculated stems were wrapped with a plastic film and incubated at 25 °C for 10 days.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from 7-day old colonies grown on PDA with cellophane using a modified CTAB method (Doyle & Doyle 1990). DNA fragment size was checked with electrophoresis on 1% agarose gels, and the concentration was measured using a Thermo NanoDrop™ 2000 according to the user manual (Desjardins & al. 2009).

For the amplification of ITS, TEF1, and TUB2, we employed a PTC-200 DNA Engine Peltier Thermal Cycler and primer pairs ITS5/ITS4 (White & al. 1990), BT2A/BT2B (Glass & Donaldson 1995, O'Donnell & Cigelnik 1997), 728F/EF2 (O'Donnell & Cigelnik 1997, Carbone & Kohn 1999). The PCR amplification products were inspected visually by electrophoresis in 2% agarose gels. DNA was sequenced using an ABI PRISM® 3730XL DNA Analyzer with BigDye® Terminator Kit v.3.1 at the Shanghai Invitrogen Biological Technology Company Limited in Beijing.

DNA sequence analysis

Our sequences and reference sequences obtained from GenBank (TABLE 1) were aligned using MAFFT v.6 (Katoh & Toh 2010) and edited manually using MEGA6 (Tamura & al. 2013). The alignments were concatenated for phylogenetic analyses. Maximum parsimony (MP) analysis was conducted with PAUP v.4.0b10 (Swofford 2003), maximum likelihood (ML) analysis with PhyML v.7.2.8 (Guindon & al. 2010), and Bayesian inference (BI) with MrBayes v.3.1.2 (Ronquist & Huelsenbeck 2003). The phylogenetic tree included our new ITS, TEF1, and TUB2 sequences and sequences from ex-type specimens in recent studies (Maharachchikumbura & al. 2014, Jayawardena & al. 2016).

Pseudopestalotiopsis cocos (CBS 272.29) and *Pestalotiopsis trachicarpicola* (OP 068) were selected as the outgroup in this analysis. Trees were visualized and examined using FigTree v.1.3.1 (Rambaut & Drummond 2010).

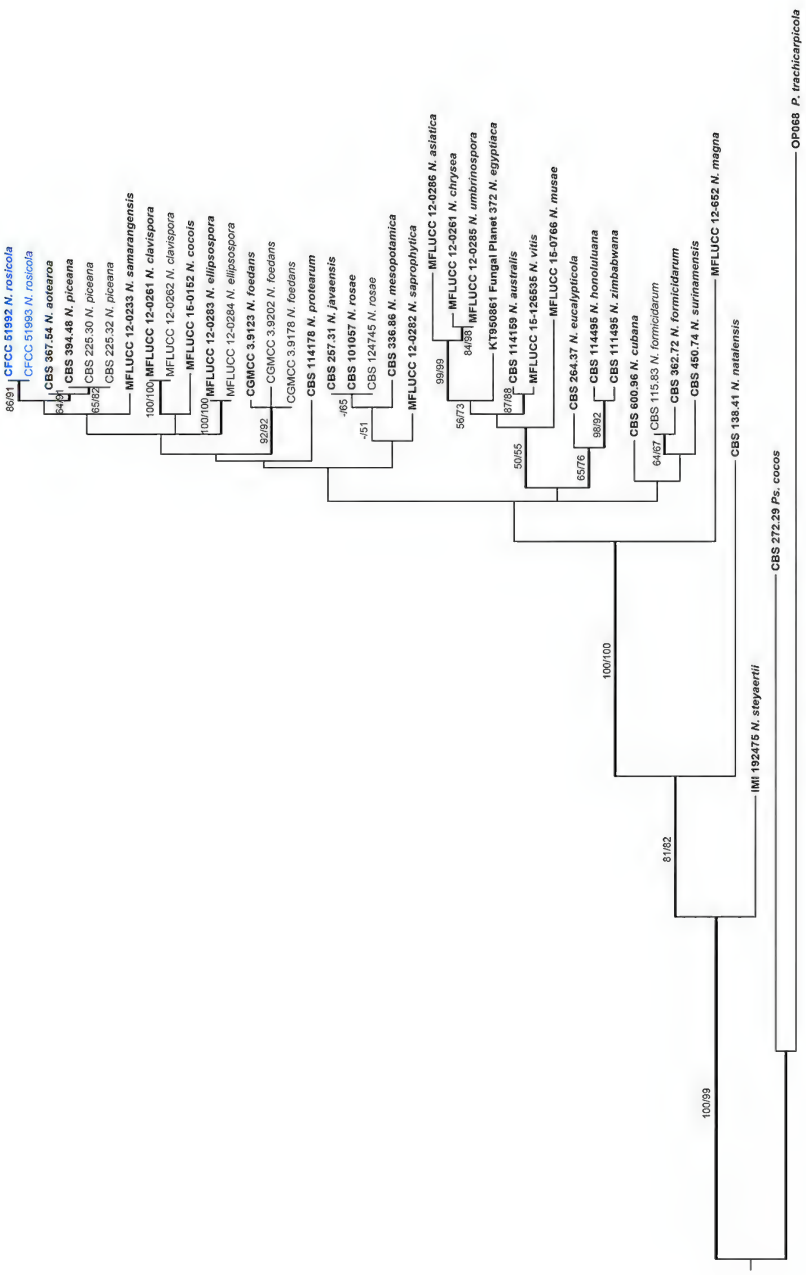
MP analysis was performed by a heuristic search option of 1000 random-addition sequences with a tree bisection and reconnection (TBR) algorithm. Maxtrees were set to 5000, branches of zero length were collapsed and all equally parsimonious trees were saved. Other calculated parsimony scores were tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency (RC). ML analysis was performed with a GTR substitution model (Guindon & al. 2010). The branch support was evaluated with a bootstrapping (BS) method of 1000 replicates (Hillis & Bull 1993). For the BI analysis, a Markov Chain Monte Carlo (MCMC) algorithm was performed (Rannala & Yang 1996). The optimal substitution model for each locus was determined with MrModeltest v.2.3 (Posada & Crandall 1998). Sequence data were deposited in GenBank (TABLE 1). The multilocus alignment file was deposited in TreeBASE (www.treebase.org) as accession 20559. The nomenclatural novelty was deposited in MycoBank (Crous & al. 2004).

Table 1. Sequences of *Neopestalotiopsis*, *Pestalotiopsis*, and *Pseudopestalotiopsis* used in the molecular analysis

Species	Isolate	Host / Substrate	Location	GenBank		
				ITS	tef1	tub2
N. aotearoa	CBS 367.54	Canvas	New Zealand	KM199369	KM199526	KM199454
N. asiatica	MFLUCC 12-0286	Unidentified tree	China	JX398983	JX399049	JX399018
N. australis	CBS 114159	*Telopea* sp.	Australia	KM199348	KM199537	KM199432
N. chrysea	MFLUCC 12-0261	Dead leaves	China	JX398985	JX399051	JX399020
N. clavispora	MFLUCC 12-0281*	*Magnolia* sp.	China	JX398979	JX399045	JX399014
	MFLUCC 12-0280	*Magnolia* sp.	China	JX398978	JX399044	JX399013
N. cocois	MFLUCC 15-0152	—	—	KX789687	KX789689	—
N. cubana	CBS 600.96	Leaf litter	Cuba	KM199347	KM199521	KM199438
N. egyptiaca	CBS 140162	*Mangifera indica*	Egypt	KP943747	KP943748	KP943746
N. ellipsospora	MFLUCC 12-0283*	Dead plant material	China	JX398980	JX399047	JX399016
	MFLUCC 12-0284	Dead plant material	Thailand	JX398981	JX399046	JX399015
N. eucalypticola	CBS 264.37	*Eucalyptus globulus*	—	KM199376	KM199551	KM199431
N. foedans	CGMCC 3.9123*	Mangrove plant	China	JX398987	JX399053	JX399022
	CGMCC 3.9178	*Neodypsis decaryi*	China	JX398989	JX399055	JX399024
	CGMCC 3.9202	*Calliandra haematocephala*	China	JX398988	JX399054	JX399023
N. formicidarum	CBS 362.72*	Dead *Formicidae*	Ghana	KM199344	KM199519	KM199444
	CBS 115.83	Plant debris	—	KM199358	KM199517	KM199455
N. honoluluana	CBS 114495	*Telopea* sp.	USA: Hawaii	KM199364	KM199548	KM199457
N. javaensis	CBS 257.31	*Cocos nucifera*	Indonesia	KM199357	KM199543	KM199437
N. magna	MFLUCC 12-652	*Pteridium* sp.	France	KF582795	KF582791	KF582793

Species	Isolate	Host / Substrate	Location	GenBank		
				ITS	tef1	tub2
N. mesopotamica	CBS 336.86	*Pinus brutia*	Iraq	KM199362	KM199555	KM199441
N. musae	MFLUCC 15-0776	—	—	KX789683	KX789685	KX789686
N. natalensis	CBS 138.41	*Acacia mollissima*	South Africa	KM199377	KM199552	KM199466
N. piceana	CBS 394.48*	*Picea* sp.	UK	KM199368	KM199527	KM199453
	CBS 254.32	*Cocos nucifera*	Indonesia	KM199372	KM199529	KM199452
	CBS 225.30	*Mangifera indica*	—	KM199371	KM199535	KM199451
N. protearum	CBS 114178	*Leucospermum cuneiforme*	Zimbabwe	JN712498	KM199542	KM199463
N. rosae	CBS 101057*	*Rosa* sp.	New Zealand	KM199359	KM199523	KM199429
	CBS 124745	*Paeonia suffruticosa*	USA	KM199360	KM199524	KM199430
N. rosicola	**CFCC 51992***	***Rosa chinensis***	**China**	**KY885239**	**KY885243**	**KY885245**
	CFCC 51993	***Rosa chinensis***	**China**	**KY885240**	**KY885244**	**KY885246**
N. samarangensis	MFLUCC 12-0233	*Syzygium samarangense*	Thailand	JQ968609	JQ968611	JQ968610
N. saprophytica	MFLUCC 12-0282	*Magnolia* sp.	China	KM199345	KM199538	KM199433
N. steyaertii	IMI 192475	*Eucalyptus viminalis*	Australia	KF582796	KF582792	KF582794
N. surinamensis	CBS 450.74	Soil under *Elaeis guineensis*	Suriname	KM199351	KM199518	KM199465
N. umbrinospora	MFLUCC 12-0285	Unidentified plant	China	JX398984	JX399050	JX399019
N. vitis	MFLUCC 15-1265	*Vitis vinifera*	China	KU140694	KU140676	KU140685
N. zimbabwana	CBS 111495	*Leucospermum cuneiforme*	Zimbabwe	JX556231	KM199545	KM199456
P. trachicarpicola	OP608	*Trachycarpus fortunei*	China	JQ845947	JQ845945	JQ845946
Pseudop. cocos	CBS 272.39	*Cocos nucifera*	Indonesia	KM199378	KM199553	KM199467

New sequences are set in bold font. Ex-type/ex-epitype isolates are marked by *.


86/91 CFCC 51992 N. rosicola
CFCC 51993 N. rosicola
84/91 CBS 367.54 N. aotearoa
CBS 394.48 N. piceana
65/82 CBS 225.30 N. piceana
CBS 225.32 N. piceana
MFLUCC 12-0233 N. samarangensis
100/100 MFLUCC 12-0261 N. clavispora
MFLUCC 12-0262 N. clavispora
MFLUCC 15-0152 N. cocois
100/100 MFLUCC 12-0283 N. ellipsospora
MFLUCC 12-0284 N. ellipsospora
CGMCC 3.9123 N. foedans
92/92 CGMCC 3.9202 N. foedans
CGMCC 3.9178 N. foedans
CBS 114178 N. protearum
CBS 257.31 N. javaensis
-/65 CBS 101057 N. rosae
-/51 CBS 124745 N. rosae
CBS 336.86 N. mesopotamica
MFLUCC 12-0282 N. saprophytica
99/99 MFLUCC 12-0286 N. asiatica
MFLUCC 12-0261 N. chrysea
84/88 MFLUCC 12-0285 N. umbrinospora
56/73 KT950861 Fungal Planet 372 N. egyptiaca
87/88 CBS 114159 N. australis
MFLUCC 15-126535 N. vitis
50/55 MFLUCC 15-0766 N. musae
CBS 264.37 N. eucalypticola
65/76 98/92 CBS 114495 N. honoluluana
CBS 111495 N. zimbabwana
CBS 600.96 N. cubana
64/67 CBS 115.83 N. formicidarum
CBS 362.72 N. formicidarum
CBS 450.74 N. surinamensis
MFLUCC 12-652 N. magna
CBS 138.41 N. natalensis
100/100
81/82
IMI 192475 N. steyaertii
CBS 272.29 Ps. cocos
100/99
OP068 P. trachicarpicola
2.0.0

Results

Molecular phylogeny

The combined ITS, TEF1, and TUB2 data matrix contains 38 ingroup and two outgroup isolates. The statistics for the MP analysis revealed that of 1461 characters, 1074 characters were constant, 214 variable characters were parsimony-uninformative, and 173 characters were parsimony-informative. The MP analysis yielded one most parsimonious tree, with the tree (TL = 650, CI = 0.737, RI = 0.702, RC = 0.517) shown in FIG. 1. The phylogenetic trees obtained from ML and BI analyses were consistent with the MP tree. A novel species was identified based on the multi-locus phylogeny coupled with the morphological features described below.

Pathogenicity

On stem wounds of the treatment group, lesions developed gradually and conidiomata were produced 10 days after inoculation, while few changes were observed in the control group (FIG. 2). Newly produced conidiomata sampled from the lesions were identified as *Neopestalotiopsis rosicola* by morphological observation of conidiogenous cells and conidia.

Taxonomy

Neopestalotiopsis rosicola C.M. Tian & Ning Jiang, **sp. nov.** FIG. 3

MYCOBANK MB 820879

Differs from *Neopestalotiopsis rosae* by its shorter and less numerous tubular apical appendages.

TYPE: China, Jiangsu Prov., Gaoyou City, 32°47′25″N 119°28′12″E, 1 m asl, on twigs and branches of *Rosa chinensis* Jacq., 12 Feb 2017, coll. N. Jiang (**Holotype,** BJFC-S1386; ex-type culture CFCC 51992; GenBank KY885239, KY885243, KY885245).

ETYMOLOGY: *rosicola* (Lat.), named after the host genus, *Rosa*.

ON PDA: CONIDIOMATAL PYCNIDIA globose to oval, solitary or confluent, semi-immersed, black, 100–550 µm diam; exuding globose, black, glistening, conidial masses. CONIDIOPHORES indistinct, often reduced to conidiogenous cells. CONIDIOGENOUS CELLS discrete, cylindrical to subcylindrical, hyaline, rugose-walled, 5–20 × 2–7 µm, apex 1–5 µm diam. CONIDIA fusoid, ellipsoid, straight to slightly curved, 4-septate, (18.9–)20.2–25.5(–26.2) ×

FIG. 1 (at left). Phylogram of *Neopestalotiopsis* based on combined ITS, TEF1, and TUB2 data. MP and ML bootstrap support values >50% are shown at the first and second position. Thickened branches represent BI posterior probabilities >0.90. Scale bar = 20 nucleotide substitutions. Ex-type strains are in bold and the new species is in blue.

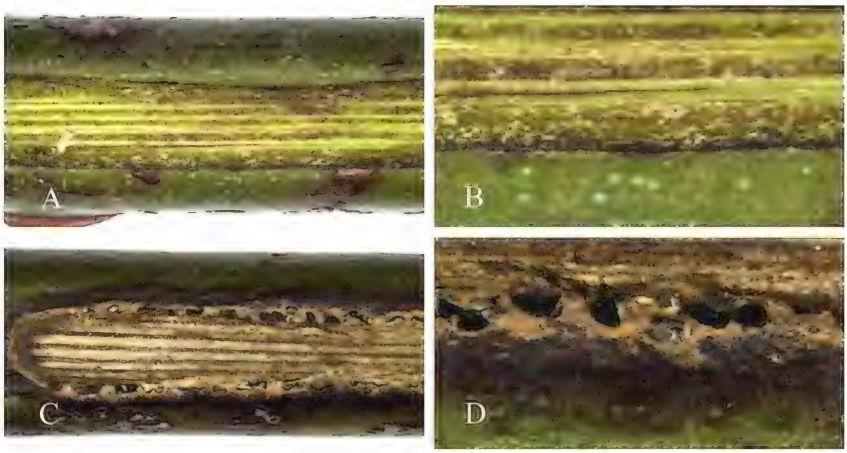


Fig. 2. *Neopestalotiopsis rosicola*: pathogenicity tests on *Rosa chinensis* stems. A, B: Control treatment wounds 10 days after being covered by pure PDA. C, D: Pathogen treatment wounds 10 days after inoculation, showing numerous sclerotia (and with conidiomata also formed).

(5–)5.5–8(–8.5) µm, mean ± SD = 22.9 ± 0.8 × 6.5 ± 0.5 µm; basal cell conic with a truncate base, hyaline, thin-walled, 2.9–5.8 µm long; three median cells doliiform, (10.7–)12.7–16.3(–18.6) µm long, mean ± SD = 10.5 ± 0.8 µm, wall rugose, versicoloured, septa darker than rest of cell; second cell from base pale brown to olivaceous, 3–6 µm long; third cell honey brown, 4.3–6.1 µm long; fourth cell brown, 3.4–6.5 µm long; apical cell 3–5 µm long, hyaline, cylindrical to subcylindrical, thin and smooth walled, with 2–4 tubular apical appendages (mostly 2 and 3, seldom 4), arising from apical crest, flexuous, unbranched, (16.5–)17–22.8(–25.9) µm long, mean ± SD = 20 ± 0.9 µm; basal appendage single, tubular, unbranched, centric, 2–9.5 µm long. Colonies on PDA attaining 40–70 mm diam after 7 days at 25°C, with undulate margin, whitish; after 15 days at 25°C, black conidiomata distributed irregularly on surface.

Host/Distribution: on branches of *Rosa chinensis* in China.

Additional specimen examined: **CHINA, Jiangsu Prov., Gaoyou City**, 32°47′25″N 119°28′12″E, 1 m asl, on twigs and branches of *Rosa chinensis* Jacq., 12 Feb 2017, coll. N. Jiang (BJFC-S1387; culture CFCC 51993; GenBank KY88240, KY885244, KY885246).

Note: The two isolates of *Neopestalotiopsis rosicola* cluster in one supported clade (MP/ML/BI = 86/91/1), closely related to *N. aotearoa* Maharachch. & al. isolated from canvas and *N. piceana* Maharachch. & al. on *Picea* sp. (Fig. 1). The new species differs from *N. aotearoa* by its longer and more

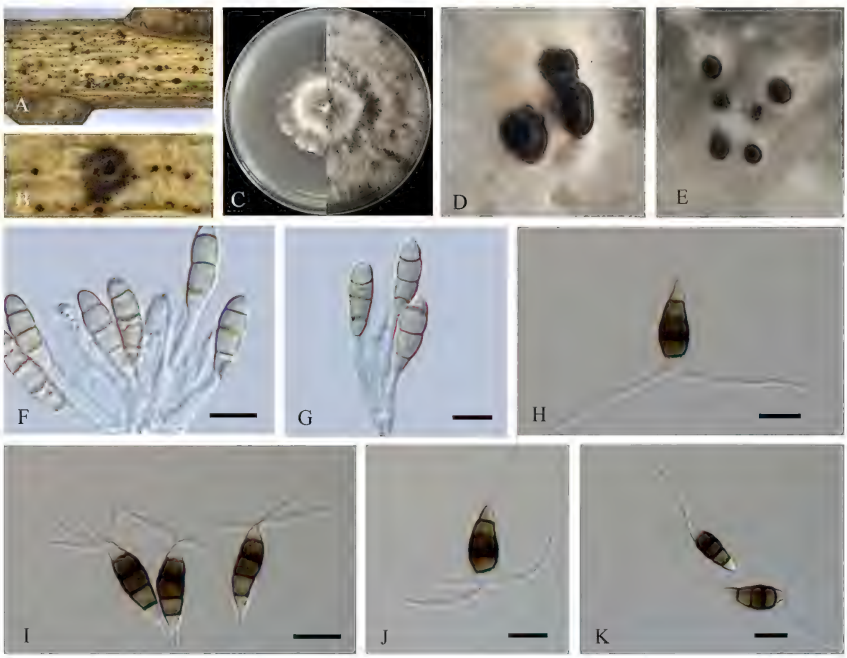


FIG. 3. *Neopestalotiopsis rosicola* (holotype, BJFC-S1386). A, B: Habit of conidiomata on *Rosa chinensis* stem. C: Colonies on PDA at 3 days (left) and 15 days (right). D, E: Conidiomata on PDA. F, G: Conidiogenous cells. H–K: Mature conidia. Scale bars: F–K = 10 µm.

numerous tubular apical appendages and from *N. piceana* by shorter and more numerous tubular apical appendages (TABLE 2).

Discussion

Neopestalotiopsis rosicola associated with a canker disease of *Rosa chinensis* in Jiangsu Province, China, is introduced as a new species based on morphological characteristics and molecular data (ITS, TEF1, and TUB2). It is distinguished from two *Neopestalotiopsis* species previously described from *Rosa*. *Neopestalotiopsis rosae* causing stem lesions on *Rosa* sp. in New Zealand (Maharachchikumbura & al. 2014) is morphologically distinct from *N. rosicola* (FIG. 1; TABLE 2) and produces only a few conidiomata on the diseased host tissue. *Neopestalotiopsis clavispora,* causing rose leaf blotch in China (Feng & al. 2014), differs from *N. rosicola* by its longer and less numerous tubular apical appendages (TABLE 2). The three species on *Rosa* clustered in separate clades in the combined *Neopestalotiopsis* phylogeny (FIG. 1).

Table 2. Comparison of *Neopestalotiopsis rosicola* and related species

Species	Strain	Conidia (μm)	Apical appendages		Host/Substrate	Location	Reference
			Number	Length (μm)			
N. aotearoa	CBS 367.54	21–28 × 6.5–8.5	2–3	5–12	Canvas	New Zealand	Maharachchikumbura & al. 2014
N. clavispora	YJYK-1	18–26 × 6–8.5	2–3	19–32	*Rosa chinensis*	China	Maharachchikumbura & al. 2012; as *Pestalotiopsis clavispora*
N. piceana	CBS 394.48	19.5–25 × 7.5–9	3	21–31	*Picea* sp.	UK	Maharachchikumbura & al. 2014
N. rosae	CBS 101057	22–37 × 7.5–9.5	3–5	24–31	*Rosa* sp.	New Zealand	Maharachchikumbura & al. 2014
N. rosicola	**CFCC 61992**	**20.2–25.5 × 5.5–8**	**2–4**	**17–23**	***Rosa chinensis***	**China**	This paper

The new species is set in bold font.

Neopestalotiopsis rosicola was verified as the pathogen causing China rose canker disease in our pathogenicity test. However, preliminary experiments with mycelium from *N. rosicola* (data not shown) indicated that direct infection of the host is unlikely. Considering that these ornamental plants are regularly pruned, we inoculated artificially induced wounds. *Neopestalotiopsis rosicola* was shown to infect wounded hosts quickly, producing conidiomata in 10 days and capable of being re-isolated from the diseased host. We suggest that gardeners adopt protective measures during pruning, especially in Jiangsu Province.

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Distribution of *Alternaria* species among sections. 5. Species producing conidia with many longitudinal septa

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Abstract—Morphological examination of material conforming to the morphological descriptions of *Alternaria* species producing conidia with many longitudinal septa (but not phylogenetically characterized) allowed the inclusion of additional species in *Alternaria* sect. *Alternaria* (4 spp.; total spp. = 66), *A.* sect. *Cheiranthus* (1 sp.; total spp. = 3), *A.* sect. *Gypsophilae* (1 sp.; total spp. = 9), *A.* sect. *Infectoriae* (2 spp.; total spp. = 36), and *A.* sect. *Panax* (2 spp.; total spp. = 7). A complete list of species included in *Alternaria* sections *Cheiranthus*, *Gypsophilae*, and *Panax* is presented. Sixteen species were found to have intermediate morphology and could not be unambiguously assigned to any section.

Key words— *Alternaria asphodeli*, *Alternaria latifunda*, *Alternaria longispora*, *Alternaria prasonis*

Introduction

A recent series of large-scale works assessing morphology and molecular phylogeny of 13 generic alternarioid hyphomycetes has resulted in the elevation of 27 sections in the genus *Alternaria* (Lawrence & al. 2013, 2016; Woudenberg & al. 2013, 2014; Grum-Grzhimaylo & al. 2016). Many species of the enlarged genus have been assigned to these sections via morphological and/or molecular data. However, a number of *Alternaria* spp. have been described from herbarium specimens only and have no known living isolates. These species can be taxonomically considered using conserved morphological characters of the sporulation apparatus.

This work is a continuation of the series of articles dedicated to the morphological examination of *Alternaria* species that have not been assigned to any section using molecular data. Previous works assessed species with morphological features shared with existing members in *Alternaria* sect. *Porri* (Gannibal 2015), *A.* sect. *Alternaria* (Gannibal 2016), *A.* sect. *Infectoriae* and *A.* sect. *Pseudoalternaria* (Gannibal & Lawrence 2017), and *A.* sect. *Alternantherae* (species of which were previously considered to reside in the genus *Nimbya*) and *Nimbya* (Gannibal 2018). In this study we examined species that form elongated obclavate or ellipsoidal conidia with large numbers of longitudinal and oblique septa (1–2 or even 2–4 in most of the transverse segments).

Three sections, *Alternaria* sect. *Cheiranthus*, *A.* sect. *Gypsophilae*, and *A.* sect. *Panax*, are excellent candidates to serve as a "harbor" for species that produce elongated obclavate or ellipsoidal conidia with many longitudinal and oblique septa. Some species in sections *Alternaria* sect. *Alternaria*, *A.* sect. *Infectoriae*, and *A.* sect. *Porri* may also form elongated conidia with many longitudinal septa.

Alternaria sections *Cheiranthus*, *Gypsophilae*, and *Panax* are morphologically similar. All three sections possess species that form branched conidial chains of mostly 3–5 to 5–7 units. Mature conidia are of medium size (in most cases 50–150 × 15–25 µm), ellipsoidal or obclavate with several longitudinal and oblique septa in many or almost all transverse segments. Species that produce small conidia usually form fewer longitudinal septa (e.g., *Alternaria juxtiseptata*, *A. eryngii*). Lawrence & al. (2016) emended the description of *A.* sections *Cheiranthus*, *Gypsophilae*, and *Panax*. Furthermore, some minor morphological features should be mentioned. In combination with commonly used morphological characters these nonessential characters as well as ecological features can help to determine affiliation of a species with a section of *Alternaria*. For example: *A.* sect. *Panax* conidia more often form multiple lateral secondary conidiophores. *Alternaria* sect. *Gypsophilae* conidia have conspicuous constrictions near almost all septa; additionally, species in this section seem to have a strong plant host association (with four genera of *Caryophyllaceae*, tribe *Caryophylleae*) (Simmons 2002). It is challenging to define characteristic features for *A.* sect. *Cheiranthus* since it currently has only two morphologically rather divergent members.

Some species of other sections can resemble *A.* sections *Cheiranthus*, *Gypsophilae*, and *Panax* via several characters. A significant number of such species have been described from natural substrates: herbarium materials or plant parts placed in moist chamber. For example, conidia of several species in

A. sect. *Porri* (e.g., *A. cyamopsidis*, *A. euphorbiae*, *A. latispora*) have numerous longitudinal septa. Simmons (2007) has described some large-spored species from both herbarium specimens and living cultures. For each of *Alternaria solani* and *A. subcylindrica,* herbarium and culture conidia are similar. However, field conidia of *A. hibiscinficiens* were 1.5–2 times wider, did not form filiform beaks, and had more longitudinal septa (1–3 in most of transverse segments) in comparison with typical *A.* sect. *Porri* conidia produced in a moist chamber (Simmons 2007). Some other species in *A.* sect. *Porri* (e.g., *A. tagetica* and *A. sauropodis*) may produce conidia with several lateral secondary conidiophores, which is a general character for species in *A.* sect. *Panax*.

Species in *A.* sect. *Alternaria*, *A. tenuissima* and *A. citri*, can produce conidia with many longitudinal septa. Similar to *A. hibiscinficiens* in *A.* sect. *Porri*, *A. tenuissima* and *A. caudata* herbarium conidia are wider and have more intensive septation than conidia from culture on potato-carrot agar – PCA (Simmons 2007). Conidia of *A. citri* sampled from nature are also wider and have many more septa than conidia derived from colonies cultured on PCA medium. *Alternaria toxicogenica* under culture conditions has the largest conidia with the highest number of septa among *A.* sect. *Alternaria* members.

Alternaria triticina and *A. ventricosa* are examples of species in *A.* sect. *Infectoriae* with relatively large conidia with many longitudinal septa. *Alternaria triticimaculans* was studied by Simmons (2007) utilizing field specimens and cultured isolates (Simmons 2007). Conidia from herbarium samples were wider and had more intensive septation than those from culture as in the previous examples.

Simmons (2007) made some suggestions and linked some "strange" species with morphologically more well-described species or species-groups. Sometimes he combined a group of species that are now currently members of different phylogenetically validated sections. However, Simmons' viewpoint on species groupings differs somewhat as compared to the molecular phylogenetic studies and section descriptions that came much later (Lawrence & al. 2016).

The aim of this work was to assign *Alternaria* species that form elongated obclavate or ellipsoidal conidia with large numbers of longitudinal and oblique septa to sections using existing morphological descriptions.

Materials & methods

The morphology of all *Alternaria* species with legitimate names was analyzed with regard to conformity with criteria of *Alternaria* sections *Cheiranthus*, *Gypsophilae*, and *Panax*. The morphological assessment was based on protologue diagnoses and on illustrations published by Simmons (2007).

Results & discussion

Twenty-six species resembling *Alternaria* sections *Cheiranthus*, *Gypsophilae*, and *Panax* were found among unassembled *Alternaria* species. The evaluation of all possible sectional connections resulted in ten additional species being connected with known sections. Nine of these ten species have no known living isolates. Suggestions by Simmons (2007) on species similarity were not enough to link a species to any phylogenetically recognized group.

Only four additional species were recognized as belonging to *A.* sections *Cheiranthus*, *Gypsophilae*, or *Panax*. All *Alternaria* species in those three sections are listed in Table 1. Other species represent previously revised *A.* sections *Alternaria* and *Infectoriae* (Gannibal 2016; Gannibal & Lawrence 2017) or cannot be morphologically assigned to a section.

One additional species, *A. longispora*, was added to *A.* sect. *Gypsophilae*. *Alternaria longispora* was isolated from a host in *Caryophyllaceae*, as were all eight species previously assigned to *A.* sect. *Gypsophilae*. An additional species, *A. latifunda*, was added to *A.* sect. *Cheiranthus*, which previously comprised two morphologically divergent species.

Alternaria asphodeli and *A. prasonis* were added to *A.* sect. *Panax*, which previously comprised five species. *Alternaria prasonis* was supposed by Simmons (2007) to be a member of the infectoria species-group, where he also placed *A. avenicola* (*A.* sect. *Panax*). Only *A. prasonis* among species distributed among sections in this article has a living representative isolate (Simmons 2007).

Four species show high morphological affinity with *A.* sect. *Alternaria*. Three species have conidia similar to *A. tenuissima* field conidia (conical or subulate apex or short secondary conidiophores; obclavate shape) but somewhat larger: *A. calystegiae*, *A. diversispora*, and *A. guaranitica*. One species, *A. macalpinei*, produces conidia resembling *A. citri* field conidia (short oval or subsphaerical body) but somewhat larger. Including species previously listed (Gannibal 2016; 2018), currently 66 species fit the morphological parameters of *A.* sect. *Alternaria*.

Alternaria polytricha and *A. shaanxiensis* were added to *A.* sect. *Infectoriae*, despite Simmons' (2007) note that *A. shaanxiensis* is similar to *A. cheiranthi*, the type species of *A.* sect. *Cheiranthus*. Those species have conidial similarities with field specimens of *A. triticimaculans*. Roughly 50% of their conidia are obovoid (as in many *A.* sect. *Infectoriae* species) in contrast to the mostly obclavate conidia in *A.* sect. *Alternaria*. At the same time, many *A. polytricha* and *A. shaanxiensis* conidia are obclavate with apical secondary conidiophores or with a conical or awl-like apical cell (as in *A. tenuissima* field conidia; Simmons

TABLE 1. Section assignment of *Alternaria* species producing conidia with many longitudinal septa.

Alternaria* sect. *Cheiranthus

A. cheiranthi (Lib.) P.C. Bolle (Lawrence & al. 2013, 2014; Woudenberg & al. 2013)
A. indefessa (E.G. Simmons) Woudenb. & Crous (Lawrence & al. 2013, 2014; Woudenberg & al. 2013)
A. latifunda E.G. Simmons

Alternaria* sect. *Gypsophilae

A. axiaeriisporifera E.G. Simmons & C.F. Hill (Woudenberg & al. 2013)
A. ellipsoidea E.G. Simmons (Woudenberg & al. 2013)
A. gypsophilae Neerg. (Lawrence & al. 2013; Woudenberg & al. 2013)
A. juxtiseptata E.G. Simmons (Woudenberg & al. 2013)
A. longispora McAlpine
A. nobilis (Vize) E.G. Simmons (Hoog & Horré 2002; Lawrence & al. 2013; Woudenberg & al. 2013)
A. saponariae (Peck) Neerg. (Woudenberg & al. 2013)
A. vaccariae (Săvul. & Sandu) E.G. Simmons & S.T. Koike (Lawrence & al. 2013; Woudenberg & al. 2013)
A. vaccariicola E.G. Simmons (Lawrence & al. 2013; Woudenberg & al. 2013)

Alternaria* sect. *Panax

A. asphodeli O. Săvul.
A. avenicola E.G. Simmons, Kosiak & Kwaśna (Woudenberg & al. 2013)
A. calycipyricola R.G. Roberts (Lawrence & al. 2013)
A. eryngii (Pers.) S. Hughes & E.G. Simmons (Lawrence & al. 2013, 2014)
A. panax Whetzel (Hoog & Horré 2002; Lawrence & al. 2013, 2014; Woudenberg & al. 2013)
A. photistica E.G. Simmons (Hoog & Horré 2002; Woudenberg & al. 2013)
A. prasonis E.G. Simmons

Additions to *Alternaria* sect. *Alternaria**

A. calystegiae Nelen
A. diversispora (Thüm.) E.G. Simmons
A. guaranitica (Speg.) E.G. Simmons
A. macalpinei E.G. Simmons

Additions to *Alternaria* sect. *Infectoriae***

A. polytricha (Cooke) E.G. Simmons
A. shaanxiensis T.Y. Zhang & J.Z. Zhang

Incertae sedis

A. bannaensis W.Q. Chen & T.Y. Zhang
A. capsici-annui Săvul. & Sandu
A. ellisii Pandotra & Ganguly
A. hibiscina (Thüm.) E.G. Simmons
A. interna (McAlpine) P. Joly
A. iridicola (Ellis & Everh.) J.A. Elliott
A. limnanthemicola R.L. Mathur, Agnihotri & Tyagi
A. malvacearum E.G. Simmons
A. mycophila (Bubák & Dearn.) P. Joly
A. nerii (Cooke) E.G. Simmons
A. ornatissima (Ellis & Barthol.) P. Joly
A. papaveris (Bres.) M.B. Ellis
A. peponicola (Rabenh.) E.G. Simmons
A. putrefaciens (Fuckel) E.G. Simmons
A. triticicola V.G. Rao
A. ulmi (Fuckel) E.G. Simmons

Names with bracketed annotations = species supported by phylogenetic data (in the cited references).
Unannotated names = species assigned to any section of *Alternaria* for the first time.
* For main species list see Gannibal (2016).
** For main species list see Gannibal & Lawrence (2017).

2007). The addition of those two species to *A.* sect. *Infectoriae* (Gannibal & Lawrence 2017) enlarged the section to 36 distinct morphological species.

Morphological data on the remaining 16 species known only from herbarium specimens are not sufficient to affiliate them to a particular section. Future molecular research on the remaining herbarium material offers hope for clarifying their taxonomic position. At this moment we consider species listed below as incertae sedis within the genus *Alternaria*.

Species sharing intermediate characters of *Alternaria* sections *Alternaria*, *Infectoriae*, and *Panax* are: *A. capsici-annui*, *A. ellisii*, *A. hibiscina*, *A. interna*, *A. limnanthemicola*, *A. malvacearum*, *A. mycophila*, *A. nerii*, *A. ornatissima*, *A. papaveris*, *A. peponicola*, *A. putrefaciens*, and *A. ulmi*. Simmons (2007) noted the similarity of *A. capsici-annui* with *A. triticicola* and *A. putrefaciens*, which are also incertae sedis. Simmons noted an absence of living *A. capsici-annui* isolates that can be used as references. A non-representative isolate of '*A. capsici-annui*' (CBS 504.74) clustered within species in *A.* sect. *Ulocladium* (Woudenberg & al. 2013). This species has relatively short conidia with no lateral secondary conidiophores and commonly with a moderate number of longitudinal septa. Thus, *A. capsici-annui* should likely be affiliated with *A.* sect. *Alternaria* or *A.* sect. *Infectoriae*. An assignment of a neotype of *A. capsici-annui* would give an opportunity to test this hypothesis.

Three additional species (*A. bannaensis*, *A. iridicola*, *A. triticicola*) share characters intermediate between *A.* sect. *Panax* and *A.* sect. *Porri* and are not clearly differentiated with regard to section affiliation.

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Woudenberg JHC, Truter M, Groenewald JZ, Crous PW. 2014. Large-spored *Alternaria* pathogens in section *Porri* disentangled. Studies in Mycology 79: 1–47. https://doi.org/10.1016/j.simyco.2014.07.003

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Distribution of *Alternaria* species among sections. 6. Species formerly assigned to genus *Ulocladium*

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Abstract—Morphological examination of phylogenetically unexamined species of the superseded genus *Ulocladium* allowed for the inclusion of additional species in *Alternaria* sect. *Pseudoulocladium* (2 spp.; total spp. = 6), *A.* sect. *Ulocladioides* (3 spp.; total spp. = 20), and *A.* sect. *Ulocladium* (1 sp.; total spp. = 4). Eight new combinations and four replacement names are established to bring nomenclature in accordance with modern *Alternaria* taxonomy.

Key words—*Alternaria populicola*, *Alternaria preussii*, *Alternaria pseudobotrytis*, *Alternaria sylvestris*

Introduction

Ulocladium was first described by Preuss in 1851. However, the genus was disregarded by taxonomists for more than 100 years until it was resurrected by Simmons (1967). About 30 species have been included in *Ulocladium*.

Recently, all former alternarioid hyphomycetes (13 genera) were transferred to *Alternaria* based on extensive morphological and phylogenetic studies, including the type species of the genus *Ulocladium*, *Alternaria botrytis* (Woudenberg & al. 2013). The expanded *Alternaria* has been divided into 27 taxonomic sections (Lawrence & al. 2016).

The 35 epithets connected with *Ulocladium* represent 32 species, as three epithets are synonyms or orthographic variants of other species names. The

phylogenetic position of 26 species has been unveiled (Runa & al. 2009; Wang & al. 2009, 2010; Lawrence & al. 2013; Woudenberg & al. 2013; Geng & al. 2014). Nineteen former *Ulocladium* species have been distributed among three main sections within the enlarged genus *Alternaria* based on phylogenetic data: *A.* sect. *Pseudoulocladium*, *A.* sect. *Ulocladioides*, and *A.* sect. *Ulocladium*, consisting almost exclusively of former *Ulocladium* species. Two species were connected with other more distant sections: *U. chlamydosporum* Mouch. [≡ *A. mouchaccae* E.G. Simmons] within *A.* sect. *Phragmosporae* and *U. litoreum* Pivkin & Zvereva [≡ *A. litorea* (Pivkin & Zvereva) E.G. Simmons] within *A.* sect. *Infectoriae*. The infrageneric taxonomic position of seven species has not been formalized, and six species remain phylogenetically unexamined.

Alternaria sections *Pseudoulocladium*, *Ulocladioides*, and *Ulocladium* are morphologically similar. However, some minor morphological features (combined with archetypal features) can help determine affiliation of a species with a section of *Alternaria*: *A.* sect. *Pseudoulocladium* conidia are commonly produced in simple or branched chains; members of *A.* sect. *Ulocladioides* usually produce densely geniculate (sharply angled) conidiophores with conidial clusters, conidia sometimes form short chains, and when present, secondary conidiophores are short with several conidiogenous loci; and while morphologically very similar to *A.* sect. *Ulocladioides*, *A.* sect. *Ulocladium* typically produces single conidia or small clusters of conidia with no chains.

This work is a continuation of the series of articles dedicated to the morphological examination of *Alternaria* species that have not been assessed using molecular data. Previous works grouped species with common morphological features into nine sections (Gannibal 2015, 2016, 2018; Gannibal & Lawrence 2017, 2018).

The aims of this work were i) to examine the morphology of phylogenetically unexamined *Ulocladium* species and determine their morphological affiliation with any *Alternaria* section and ii) to formalize the taxonomic status of *Ulocladium* species with defined phylogenetic positions that have not undergone taxonomic revision in order to propose a stable and accepted taxonomy to these unique lineages within the pleomorphic genus *Alternaria*.

Materials & methods

The morphology of almost all *Alternaria* species with legitimate names was analyzed with regard to conformity with criteria of *Alternaria* sect. *Pseudoulocladium*, *A.* sect. *Ulocladioides*, and *A.* sect. *Ulocladium*. We based our morphological assessment on original diagnoses and illustrations (Simmons 1967, Yen 1981, Liu & Zhang 2008, Nagaraju & al. 2009, Shipunov & al. 2009).

Results & discussion

The evaluation of descriptions of six *Ulocladium* species allowed for placement within *Alternaria*. We place two species in *A.* sect. *Pseudoulocladium*, three species in *A.* sect. *Ulocladioides*, and one species in *A.* sect. *Ulocladium*. *Alternaria* species representing the former *Ulocladium* and their sectional *Alternaria* affiliation are listed in Table 1. As a result of this study, *A.* sect. *Pseudoulocladium* now includes six species, *A.* sect. *Ulocladioides* twenty species, and *A.* sect. *Ulocladium* four species.

To bring nomenclature in accordance with modern-day *Alternaria* taxonomy we propose eight new combinations and four replacement names.

Taxonomy

Alternaria allii-tuberosi (X.G. Zhang & T.Y. Zhang) Gannibal & D.P. Lawr., **comb. nov.**

MycoBank 822967

≡ *Ulocladium allii-tuberosi* X.G. Zhang & T.Y. Zhang, Mycosystema 25(4): 516. 2006.

Alternaria castaneae (X.G. Zhang & T.Y. Zhang) Gannibal & D.P. Lawr., **comb. nov.**

MycoBank 822968

≡ *Ulocladium castaneae* X.G. Zhang & T.Y. Zhang, Mycosystema 25(4): 517. 2006.

Alternaria gpagarwalii (Nagaraju, Kunwar, Manohar. & D.K. Agarwal) Gannibal & D.P. Lawr., **comb. nov.**

MycoBank 822966

≡ *Ulocladium gpagarwalii* Nagaraju, Kunwar, Manohar. & D.K. Agarwal, Indian Phytopathol. 62(2): 237. 2009.

Alternaria manihoticola (J.M. Yen) Gannibal & D.P. Lawr., **comb. nov.**

MycoBank 822970

≡ *Ulocladium manihoticola* J.M. Yen, Bull. Soc. Mycol. France 97(3): 132. 1981.

Alternaria microspora (Moub. & Abdel-Hafez) Gannibal & D.P. Lawr., **comb. nov.**

MycoBank 822971

≡ *Ulocladium microsporum* Moub. & Abdel-Hafez, Trans. Brit. Mycol. Soc. 69(1): 164. 1977.

Alternaria oblongo-obovoidea (X.G. Zhang & T.Y. Zhang) Gannibal & D.P. Lawr., **comb. nov.**

MycoBank 822972

≡ *Ulocladium oblongo-obovoideum* X.G. Zhang & T.Y. Zhang, Mycosystema 21(1): 25. 2002.

Alternaria populicola Gannibal & D.P. Lawr., **nom. nov.**

MycoBank 822973

≡ *Ulocladium populi* E.G. Simmons, G. Newc. & A. Shipunov, Persoonia 23: 181. 2009.
non *Alternaria populi* T.Y. Zhang 2003.

Etymology: from the Latin *Populus* (the host plant genus) and *cola* (inhabitor).

Alternaria preussii Gannibal & D.P. Lawr., **nom. nov.**

MycoBank 822974

≡ *Ulocladium dauci* E.G. Simmons, Canad. J. Bot. 76(9): 1538. 1999 ["1998"].
non *Alternaria dauci* (J.G. Kühn) Groves & Skolko 1944.

Etymology: in honour of Carl Gottlieb Traugott Preuss, who described the genus *Ulocladium*.

Alternaria pseudobotrytis Gannibal & D.P. Lawr., **nom. nov.**

MycoBank 822969

≡ *Ulocladium leve* H.M. Liu & T.Y. Zhang, Mycosystema 27(1): 1. 2008.
non *Alternaria levis* Gambogi ex E.G. Simmons 2007.

Etymology: referring to the similarity of the fungus with *Alternaria botrytis*.

Alternaria sorghi (X.G. Zhang & T.Y. Zhang) Gannibal & D.P. Lawr., **comb. nov.**

MycoBank 822975

≡ *Ulocladium sorghi* X.G. Zhang & T.Y. Zhang, Mycosystema 25(4): 518. 2006.

Alternaria sylvestris Gannibal & D.P. Lawr., **nom. nov.**

MycoBank 822976

≡ *Ulocladium lignicola* Nagaraju, Kunwar, Manohar. & D.K. Agarwal,
Indian Phytopathol. 62(2): 238. 2009.
non *Alternaria lignicola* (Corda) Fr. 1849.

Etymology: from the Latin *sylva* (forest), vegetation type where the species was first found.

Alternaria zantedeschiae (X.G. Zhang & T.Y. Zhang) Gannibal & D.P. Lawr., **comb. nov.**

MycoBank 822977

≡ *Ulocladium zantedeschiae* X.G. Zhang & T.Y. Zhang, Mycosystema 25(4): 519. 2006.

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TABLE 1. List of *Alternaria* spp. assigned to *A.* sect. *Pseudoulocladium*, *A.* sect. *Ulocladioides*, and *A.* sect. *Ulocladium*.

Alternaria* sect. *Pseudoulocladium

A. aspera Woudenb. & Crous (Woudenberg & al. 2013)
≡ *U. arborescens* E.G. Simmons

A. chartarum Preuss (Runa & al. 2009; Wang & al. 2009, 2010; Lawrence & al. 2013; Woudenberg & al. 2013)
≡ *U. chartarum* (Preuss) E.G. Simmons

A. concatenata Woudenb. & Crous (Wang & al. 2009, 2010; Woudenberg & al. 2013)
≡ *U. capsici* F. Xue & X.G. Zhang [as "*capsicuma*"]

A. lanuginosa (Harz) Sacc.
≡ *Stemphylium lanuginosum* Harz
≡ *U. lanuginosum* (Harz) E.G. Simmons

A. septospora (Preuss) Woudenb. & Crous (Runa & al. 2009; Wang & al. 2010; Lawrence & al. 2013; Woudenberg & al. 2013)
≡ *Helminthosporium septosporum* Preuss
≡ *U. septosporum* (Preuss) E.G. Simmons

A. sylvestris Gannibal & D.P. Lawr.
≡ *U. lignicola* Nagaraju, Kunwar, Manohar. & D.K. Agarwal

Alternaria* sect. *Ulocladioides

A. allii-tuberosi (X.G. Zhang & T.Y. Zhang) Gannibal & D.P. Lawr. (Geng & al. 2014)
≡ *U. allii-tuberosi* X.G. Zhang & T.Y. Zhang

A. atra (Preuss) Woudenb. & Crous (Runa & al. 2009; Wang & al. 2009, 2010; Lawrence & al. 2013; Woudenberg & al. 2013)
≡ *U. atrum* Preuss

A. brassicae-pekinensis Woudenb. & Crous (Wang & al. 2010; Woudenberg & al. 2013)
≡ *U. brassicae* Yong Wang bis & X.G. Zhang

A. cantlous (Yong Wang bis & X.G. Zhang) Woudenb. & Crous (Woudenberg & al. 2013; Geng & al. 2014)
≡ *U. cantlous* Yong Wang bis & X.G. Zhang

A. castaneae (X.G. Zhang & T.Y. Zhang) Gannibal & D.P. Lawr. (Geng & al. 2014)
≡ *U. castaneae* X.G. Zhang & T.Y. Zhang

A. consortialis (Thüm.) J.W. Groves & S. Hughes (Runa & al. 2009; Wang & al. 2009; Lawrence & al. 2013; Woudenberg & al. 2013)
≡ *Macrosporium consortiale* Thüm.
≡ *U. consortiale* (Thüm.) E.G. Simmons

A. cucurbitae Letendre & Roum. (Runa & al. 2009; Wang & al. 2009, 2010; Lawrence & al. 2013; Woudenberg & al. 2013)
≡ *U. cucurbitae* (Letendre & Roum.) E.G. Simmons

A. gpagarwalii (Nagaraju, Kunwar, Manohar. & D.K. Agarwal) Gannibal & D.P. Lawr.
≡ *U. gpagarwalii* Nagaraju, Kunwar, Manohar. & D.K. Agarwal

A. heterospora Woudenb. & Crous (Wang & al. 2009; Woudenberg & al. 2013; Geng & al. 2014)
≡ *U. solani* Yong Wang bis & X.G. Zhang

A. microspora (Moub. & Abdel-Hafez) Gannibal & D.P. Lawr. (Geng & al. 2014)
≡ *U. microsporum* Moub. & Abdel-Hafez

A. multiformis (E.G. Simmons) Woudenb. & Crous (Runa & al. 2009; Wang & al. 2009, 2010; Lawrence & al. 2013; Woudenberg & al. 2013)
≡ *U. multiforme* E.G. Simmons

A. oblongo-obovoidea (X.G. Zhang & T.Y. Zhang) Gannibal & D.P. Lawr. (Geng & al. 2014)
≡ *U. oblongo-obovoideum* X.G. Zhang & T.Y. Zhang

A. obovoidea (E.G. Simmons) Woudenb. & Crous (Runa & al. 2009; Wang & al. 2009, 2010; Lawrence & al. 2013; Woudenberg & al. 2013)
≡ *U. obovoideum* E.G. Simmons

A. populicola Gannibal & D.P. Lawr.
≡ *U. populi* E.G. Simmons, G. Newc. & A. Shipunov

A. preussii Gannibal & D.P. Lawr. (Runa & al. 2009; Wang & al. 2009, 2010; Lawrence & al. 2013)
≡ *U. dauci* E.G. Simmons

A. pseudobotrytis Gannibal & D.P. Lawr.
≡ *U. leve* H.M. Liu & T.Y. Zhang

A. sorghi (X.G. Zhang & T.Y. Zhang) Gannibal & D.P. Lawr. (Geng & al. 2014)
≡ *U. sorghi* X.G. Zhang & T.Y. Zhang

A. subcucurbitae (Yong Wang bis & X.G. Zhang) Woudenb. & Crous (Wang & al. 2009, 2010; Woudenberg & al. 2013; Geng & al. 2014)
≡ *U. subcucurbitae* Yong Wang bis & X.G. Zhang

A. terricola Woudenb. & Crous (Runa & al. 2009; Lawrence & al. 2013; Woudenberg & al. 2013)
≡ *U. tuberculatum* E.G. Simmons

A. zantedeschiae (X.G. Zhang & T.Y. Zhang) Gannibal & D.P. Lawr. (Geng & al. 2014)
≡ *U. zantedeschiae* X.G. Zhang & T.Y. Zhang

Alternaria sect. *Ulocladium*

A. alternariae (Cooke) Woudenb. & Crous (Runa & al. 2009; Woudenberg & al. 2013)
≡ *Sporidesmium alternariae* Cooke
≡ *U. alternariae* (Cooke) E.G. Simmons

A. botrytis (Preuss) Woudenb. & Crous (Woudenberg & al. 2013)
≡ *U. botrytis* Preuss

A. manihoticola (J.M. Yen) Gannibal & D.P. Lawr.
≡ *U. manihoticola* J.M. Yen

A. oudemansii (E.G. Simmons) Woudenb. & Crous (Runa & al. 2009; Woudenberg & al. 2013)
≡ *U. oudemansii* E.G. Simmons

Alternaria sp. [strain CBS 504.74, misidentified as '*A. capsici-annui*' Săvul. & Sandu] (Woudenberg & al. 2013)

Names with bracketed annotations = species with phylogenetic data (in the cited references).

Unannotated names = species assigned to a section of *Alternaria* in this article for the first time.

Names in bold = new combinations or replacement names.

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Gannibal PhB. 2018. Distribution of *Alternaria* species among sections. 4. Species formerly assigned to genus *Nimbya*. Mycotaxon 133(1): 37–43. https://doi.org/10.5248/133.37

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Runa F, Park MS, Pryor BM. 2009. *Ulocladium* systematics revisited: phylogeny and taxonomic status. Mycological Progress 8: 35–47. https://doi.org/10.1007/s11557-008-0576-y

Shipunov A, Raghavendra AK, Ganley RJ, Newcombe G. 2009. Fungal Planet 34 – 23 December 2009. *Ulocladium populi* E.G. Simmons, G. Newcombe & A. Shipunov, sp. nov. Persoonia 23: 180–181. http://www.fungalplanet.org/content/pdf-files/FP34.pdf

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Endophragmiella terricola, *Gliomastix verrucipes*, and *Radulidium guttiforme* spp. nov. from soil in China

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Abstract—Three new species from soil in China—*Endophragmiella terricola*, *Gliomastix verrucipes*, *Radulidium guttiforme*—are described, illustrated, and compared morphologically with similar species. The holotype specimens (dried cultures) and living cultures are deposited in the Herbarium of Shandong Agricultural University: Plant Pathology (HSAUP).

Key words—anamorphic fungi, dematiaceous hyphomycete, morphology, taxonomy

Introduction

Three undescribed species were obtained during a survey of soil dematiaceous hyphomycete diversity in China. The morphological characteristics of the fungi are described, illustrated, and compared with similar taxa. Dried culture specimens and living cultures are conserved in the Herbarium, Department of Plant Pathology, Shandong Agricultural University, Taian, China (HSAUP).

Endophragmiella terricola J.J. Xu & T.Y. Zhang, **sp. nov.** Fig. 1

MycoBank MB 823960

Differs from *Endophragmiella suttonii* by its bigger, 0–3-septate conidia with thin septa.

§ Y.L. Jiang and Y.M. Wu contributed equally to this work.

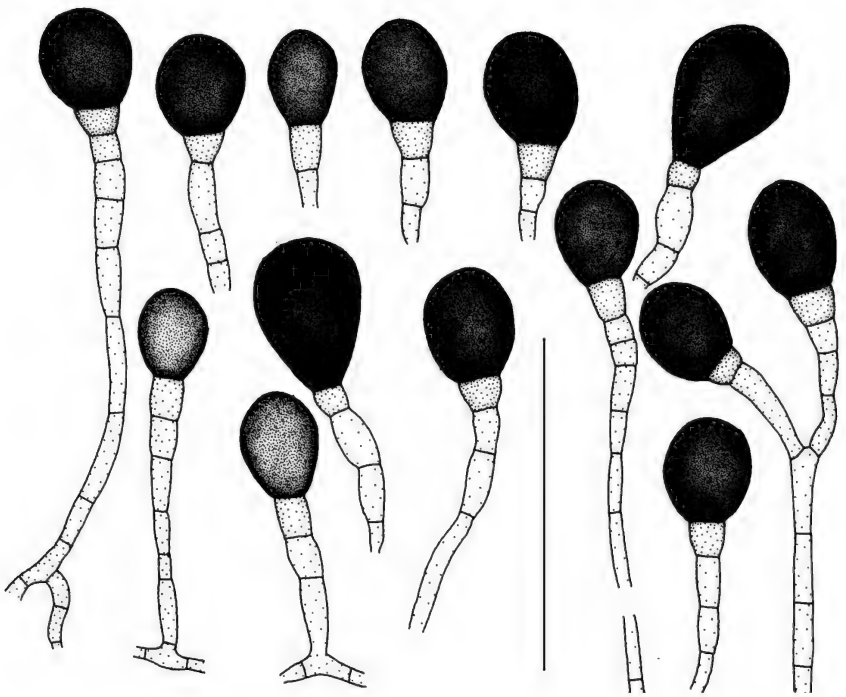

Fig. 1. *Endophragmiella terricola* (ex holotype, HSAUP $II_{04}2767$). Conidia, conidiophores, and conidiogenous cells. Scale bar = 50 μm.

Type: China, Guangdong Province, Zhanjiang City, from botanical garden soil, August 2004, J.J. Xu (**Holotype**, HSAUP $II_{04}2767$; ex-type culture, HSAUP $II_{04}2767$).

Etymology: in reference to the habitat.

Colonies on PDA effuse, sparsely hairy, at first white, later grey to dark grey, reverse pale brown, growing rapidly, reaching a diameter of 3–7.5 cm in 2 weeks at 25 °C. Mycelium mostly superficial; hyphae septate, branched, smooth, hyaline to pale brown, 1.5–4 μm diam. Conidiophores lateral or terminal, erect or ascending, septate, simple or occasionally branched, constricted at the base which connected with the hyphae, thickened to the apex, subhyaline to pale brown, 13–100 × 2.5–5 μm. Conidiogenous cells monoblastic, terminal, subhyaline, pale brown to brown, percurrent, obtrapezoidal, cylindrical or oblong, 5–13 × 1.5–6 μm. Conidia acrogenous, smooth, subspherical, pyriform or broadly clavate, 0–3-septate, apical cell brown to dark brown and swollen to ⅔–¾ of the whole spore, sometimes

thick-walled, tapering abruptly to the subapical cell, basal cell (or cells) small and subhyaline to pale brown, 18–22 × 12.5–16.5 µm.

Comments: The conidia of *Endophragmiella terricola* are most similar to those of *E. suttonii* P.M. Kirk. However, in *E. suttonii* the conidia are smaller (16–18 × 9.5–11 µm), and 3–4-septate with two thick distal septa (Kirk 1981).

Gliomastix verrucipes T.Y. Zhang, Y.M. Wu & J.J. Xu, **sp. nov.** Fig. 2

MycoBank MB 823962

Differs from *Gliomastix luzulae* by its shorter, ovoid or ellipsoidal conidia.

Type: China, Fujian Province, Wuyi Mountains, from bamboo forest soil, August 2004, J.J. Xu (**Holotype**, HSAUP $II_{04}2773$; ex-type culture, HSAUP $II_{04}2773$; **isotype**, HMAS 196295).

Etymology: in reference to the verruculose phialides.

Colonies on PDA effuse, at first white, finally greyish black or dark green, floccose or powdery, growing moderately fast, reaching 5–7 cm in diameter

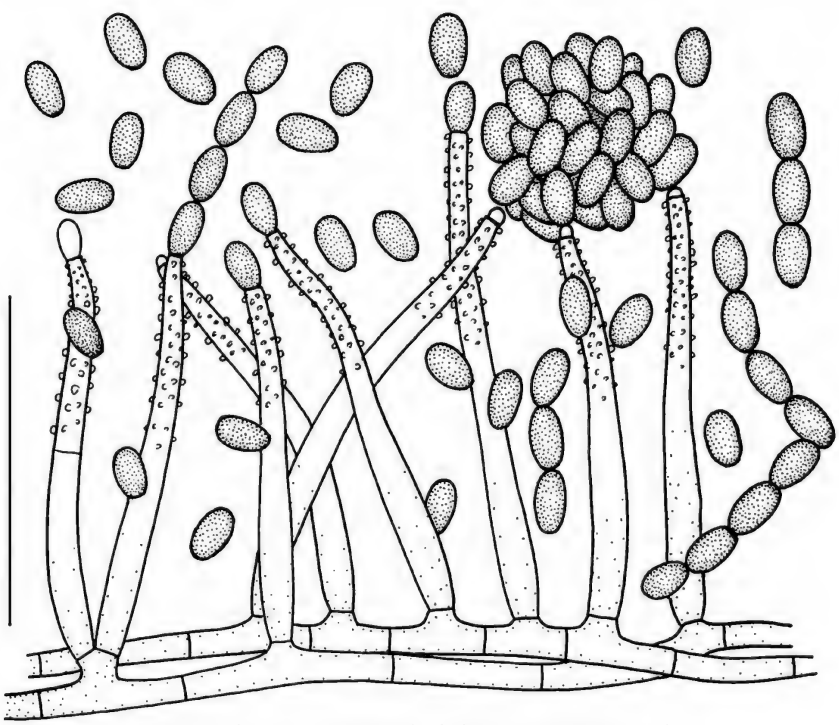

Fig. 2. *Gliomastix verrucipes* (ex holotype, HSAUP $II_{04}2773$). Conidia, hyphae, and phialides. Scale bar = 25 µm.

in 2 weeks at 25 °C. Mycelium mostly superficial; hyphae smooth, hyaline or rather pale brown, septate, branched, 1–2 µm diam. Phialides solitary or in fascicles, straight or slightly curved, verruculose above the middle part, hyaline or subhyaline, cylindrical or subulate, unbranched, 24–35 × 2–2.5 µm, swollen at the base to 2.5–3 µm. Conidia unicellular, obovate or ellipsoidal, truncate or rounded at the base, greenish brown, smooth, 4–6 × 2.5–3 µm.

Comments: *Gliomastix verrucipes* is morphologically similar to *G. luzulae* E.W. Mason ex S. Hughes in having subulate phialides roughened, at least in the middle and upper parts, but *G. luzulae* differs by its longer or narrower (4.5–9.3 × 1.5–2.9 µm, mostly 6.7 × 2.3 µm) and fusoid conidia (Hughes & Dickinson 1968).

Radulidium guttiforme T.Y. Zhang, Y.M. Wu & J.H. Kong, **sp. nov.** Fig. 3

MycoBank MB 823963

Differs from previously described *Radulidium* species by its droplet-like or obovate, thick-walled conidia.

Type: China, Gansu Province, Lanzhou City, Yellow River, from beach soil, August 2005, J.H. Kong (**Holotype**, HSAUP II_{05}2633; ex-type culture, HSAUP II_{05}2633).

Etymology: in reference to the guttiform conidia.

Colonies on MEA arachnoid or floccose, felted, compact, ochraceous yellow to brown, growing rapidly. Mycelium partly superficial, partly immersed, superficial hyphae funiculose. Conidiophores terminal or lateral, simple or occasionally branched, subhyaline to pale brown, 13–38 × 2–3 µm. Conidiogenous cells polyblastic, sympodial, inflated and densely covered with tapered cylindrical denticles. Conidia holoblastic, aseptate, guttiform or obovate, rounded at the apex, papillate or slightly truncate at the base, thick-walled, finely echinulate, greenish brown, 3.5–6 × 2.5–4 µm.

Comments: There are three previously described *Radulidium* species: *R. epichloes* (Ellis & Dearn.) Arzanlou & al., which differs from *R. guttiforme* by its subhyaline, rather thin-walled, and obovoidal to fusiform conidia (Hoog & Hermanides-Nijhof 1977); *R. subulatum* (de Hoog) Arzanlou & al., which differs by its subhyaline, thin- to slightly thick-walled, and fusiform to subcylindrical conidia (Hoog & Hermanides-Nijhof 1977); and *R. xigazense* Y.M. Wu & T.Y. Zhang, which differs by its subhyaline, clavate to fusiform, and more slender conidia (5–7 × 1.5–2 µm; Wu & al. 2013).

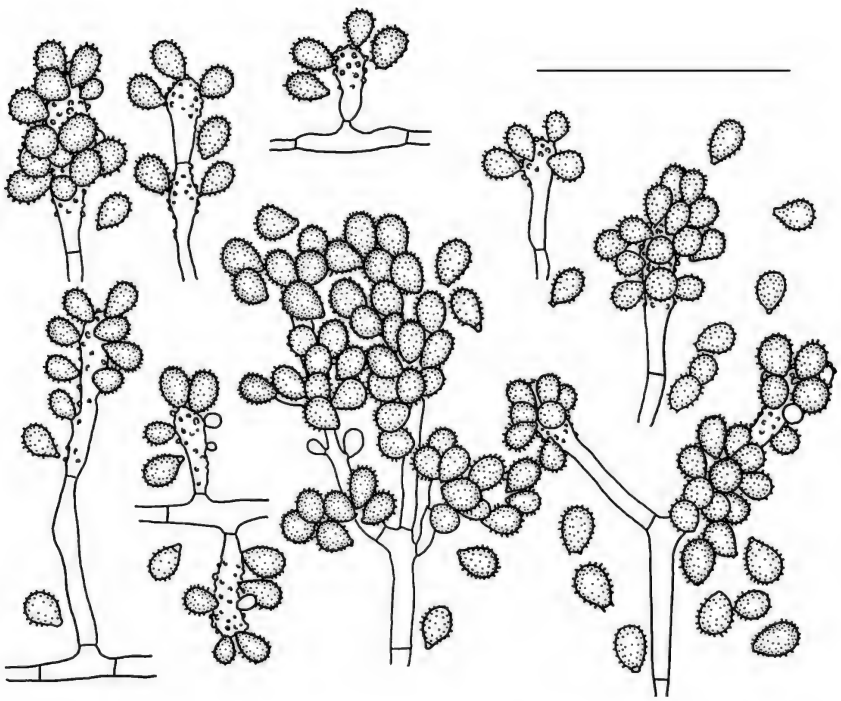

FIG. 3. *Radulidium guttiforme* (ex holotype, HSAUP $II_{05}2633$).
Conidia, conidiophores, conidiogenous cells, and denticles. Scale bar = 25 µm.

Acknowledgments

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Notes on rust fungi in China 5. Hosts and distribution of *Uromyces gageae* and its intracellular spermogonia

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Abstract—A rust fungus on *Gagea nakaiana* was collected in Jilin Province, northeast China. Based on morphological observations, specimens on this plant were identified as *Uromyces gageae*. This plant is a new host for *U. gageae* in China and the rust is newly recorded from northeast China. During field investigations orange-yellow spots were frequently observed on the leaves with rust sori. Molecular and morphological analyses confirm that those spots represent spermogonia of *U. gageae* produced in host cells.

Key words—*Liliaceae, Pucciniomycetes,* taxonomy, *Uredinales*

Introduction

Gagea nakaiana [≡ *G. lutea* var. *nakaiana*] is a perennial herb producing yellow flowers in early spring, the plant disappearing after the flowers have died. *Gagea nakaiana* is distributed in northeast China, Japan, Korea, Russian Far East, Nepal, Pakistan, Sikkim, and India (Flora of China, www.eFloras.org). There have been no previous reports of rust fungi on *G. nakaiana* in China (Tai 1979, Zhuang 2005), but in May 2016 during our investigations of rust fungi in Jilin Province, northeast China, the telial stage of a rust fungus was found on *G. nakaiana*. The rust was identified through morphological observations of the Jilin Province specimens.

Orange-yellow spots around telial sori on the leaves were also frequently observed in the specimens. These spots, which contained numerous small spermatium-like spores, were suspected to represent spermogonia of the rust fungus. Inoculation experiments with teliospores are difficult because leaves with telia are found only in early spring, and teliospores are contaminated with soil from around plants. We report here the results of our morphological observations and molecular analyses of the rust to confirm the relationship between these suspected spermogonia spots and telia.

Materials & methods

Specimens on *G. nakaiana* were collected from the forest floor in Jilin, Jilin Province, China, in May 2016 and April 2017. The deciduous broad-leaved forest, typical for the area, is composed primarily of *Betula, Populus,* and *Quercus* species. The specimens used for morphological observations and molecular analysis were deposited in the Herbarium of Mycology, Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, China (HMJAU).

Morphological characters (including the size and shape of sori and spores) were examined using light (LM) and scanning electron microscopy (SEM) as reported in Ji & al. (2017).

For molecular analyses total genomic DNA was extracted separately from a mass of spermatium-like spores from a single orange-yellow spot and about 200 spores from a single telium. Orange-yellow spots and telia were selected from separate isolated areas on the leaves to avoid cross contamination. Spores were crushed between two sterilized glass slides and suspended in 30 µl extraction buffer [10 mM Tris-HCl pH 8.3, 1.5 mM MgCl2, 50 mM KCl, 0.01% sodium dodecyl sulfate (SDS), 0.01% Proteinase K], and the suspensions were incubated at 37°C for 1 hour and 95°C for 10 min, followed by a 4°C soak (Suyama & al. 1996, Virtudazo & al. 2001). From the crude extract, 5–7 µl samples were used directly for each polymerase chain reaction (PCR). The rDNA-28S region was amplified using primers NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL4 (5′-GGTCCGTGTTTCAAGACGG-3′) (O'Donnell 1993), and the rDNA-ITS region was amplified using primers ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) (Gardes & Bruns 1993) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White & al. 1990). The DNA was amplified in a 50 µl mixture containing 5 µl of template DNA, 200 µl of each primer, 25 µl of Premix TaqTM (TaKaRa TaqTM Ver. 2.0 plus dye), and 18 µl of ddH_2O. Amplification cycling conditions consisted of 94°C for 5 min, followed by 35 denaturation cycles at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min, and a final extension at 72°C for 10 min. PCR products were separated on 1% agarose gels containing Nucleic Acid Stain (Beijing Dinggou Changsheng Biotechnology Co.) and purified using the TaKaRa MiniBEST Agarose

Fig. 1. *Uromyces gageae* on *Gagea nakaiana*. A. *Gagea nakaiana* with yellow flowers; B. Orange-yellow spots of spermogonia with sweetish exudates on a leaf; C. Vertical section of a spermogonium produced in a hypertrophied host epidermal cell; D. Dark brown telia on a leaf; E. Teliospores with hyaline papilla; F. Vertical section of a telium. Scale bars: C, E = 15 μm; F = 20 μm.

Gel DNA Exaction Kit Ver.4.0. Purified PCR products were cloned in pEASY®-T1 Cloning Vector and then transferred into Trans1-T1 phage, resistant chemically competent cell according to the Transgene Biotech instructions. The positive clones

were sequenced by Sangon Biotech Co. in Shanghai. All data sequenced in this experiment were deposited at GenBank.

Results & discussion

The telia and teliospores on *G. nakaiana* were identified as *Uromyces gageae* based on their morphological similarity to the descriptions of *U. gageae* by Gäumann (1959), Wilson & Henderson (1966), Hiratsuka & al. (1992), Azbukina (2005), and Termorshuizen & Swertz (2011), although the position of telia on the leaves and teliospore sizes differed somewhat among the descriptions. These variations may be attributable to differences in *U. gageae* populations and in host plants, given the wide range of both the rust and its host plants.

The orange-yellow leaf spots were superficially similar to spermogonia of rust fungi because of sweetish exudates from abundant minute orange-yellow spots (Fig. 1B). Spherical to hemispherical fungal structures containing small spermatia-like spores were observed inside hypertrophied epidermal cells of the leaves (Fig. 1C). Hyphal structures, including receptive hyphae, were not observed around these structures. These orange-yellow spots were also reported by Plowright in 1889, although the function of these spots was not clarified (cited from Wilson & Henderson 1966). Therefore, we conducted molecular analyses to confirm the relationship between these spots and telia of the rust fungus. Sequence data of rDNA-ITS and 28S regions were successfully obtained from single orange-yellow spots (HMJAU8557, GenBank MG742206 [ITS]; HMJAU8559, GenBank MG742208 [28S]) and single telia (HMJAU8558, GenBank MG742207 [ITS]; HMJAU8560, GenBank MG742209 [28S]). Sequence similarities of the ITS (796 bp) and 28S (652 bp) regions between the orange-yellow spot and the telium were 100%, thus confirming that the spots and telia are genetically identical and that these spots on *G. nakaiana* represent structures belonging to the rust fungus. We believe that the spots are spermogonia, although the structures differ in shape and position within the leaf tissue from previously described spermogonia (Hiratsuka & Cummins 1963, Cummins & Hiratsuka 2003), and their function is still not confirmed.

A description of the morphological features obtained from specimens on *G. nakaiana* collected in China follows.

Uromyces gageae Beck, Verh. Zool.-Bot. Ges. Wien 30: 26, 1880. Figs 1, 2

Spermogonium-like structures produced inside of hypertrophied cells amphigenous, minute, gregarious, orange-yellow, spherical to

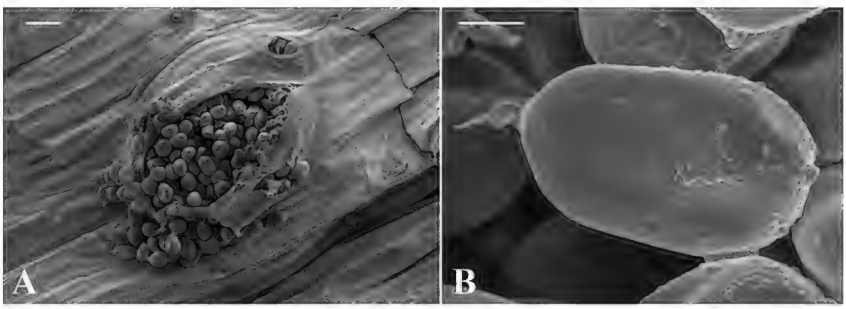

Fig. 2. *Uromyces gageae* on *Gagea nakaiana*. Telia on a leaf (A) and teliospores with small irregular verrucae (B) observed by SEM. Scale bars: A = 50 μm; B = 10 μm.

hemispherical. Aecia and uredinia not found. Telia amphigenous, dark brown to black, scattered on leaves, roundish or ellipsoid, first covered by epidermis, pulverulent, dark brown. Teliospores ellipsoid, ovate to globose, 27–39 × 19–25 μm (av. 33 × 22 μm); apex rounded with conical, hyaline papilla (ca. 2 μm); walls brown, mostly smooth, ca. 1.0–2.5 μm (av. 1.5 μm) thick, small irregular verrucae observed by SEM; pedicels hyaline, deciduous, short.

Specimens examined—Spermogonium-like structures and telia on *Gagea nakaiana* Kitag. (*Liliaceae*): **CHINA: Jilin Province, Jilin**, Jiaohe, Hongyegu, 43°42′13″N 127°04′18″E, alt. 535 m, 13 May 2016, (HMJAU8557, GenBank MG742206; HMJAU8558, GenBank MG742207); Zuojia, 44°03′51″N 126°05′54″E, alt. ca. 500 m, 21 April 2017 (HMJAU8559, GenBank MG742208); 30 April 2017 (HMJAU8560, GenBank MG742209).

Hosts & distribution in China—*Gagea nakaiana* [≡ *G. lutea* var. *nakaiana* (Kitag.) Q.S. Sun], Jilin Province; *Gagea triflora* (Ledeb.) Schult. & Schult.f. [≡ *Lloydia triflora* (Ledeb.) Baker], Inner Mongolia.

Uromyces gageae has been reported on *Gagea arvensis* (Pers.) Dumort., *G. bohemica* (Zauschn.) Schult. & Schult.f., *G. liottardii* (Sternb.) Schult. & Schult.f., *G. lutea* (L.) Ker Gawl., *G. pusilla* (F.W. Schmidt) Sweet, *G. soleirolii* F.W. Schultz, *G. spathacea* (Hayne) Salisb., and *Lloydia triflora* from Europe (Gäumann1959, Wilson & Henderson 1966, Termorshuizen & Swertz 2011); on *G. nakaiana*, *G. pauciflora* (Turcz. ex Trautv.) Ledeb., and *L. triflora* from Russian Far East (Azbukina 2005); and on *G. lutea*, and *L. triflora* from Japan (Hiratsuka & al. 1992). This rust was reported from China for the first time on *L. triflora* from Inner Mongolia (Yang & al. 2018), and *G. nakaiana* is reported here as a new host plant for China. Because *Gagea* species are

widely distributed in northern China, we suspect that *U. gageae* is also widely distributed in the area.

In our field survey, *U. gageae* was observed to produce orange-yellow spots and telia on the new young leaves when they appeared in early spring; these sori became scattered over the whole leaf surface as the leaves expanded. After producing flowers and seeds in early spring, the plant disappears until next year. However, *U. gageae* produces sori on the same individual plants in every year. We therefore suspect that *U. gageae* survives in the plant bulbs and systemically infects the new leaves as they emerge.

Acknowledgments

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Craspedodidymum guatemalense sp. nov. from Guatemala

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Abstract—*Craspedodidymum guatemalense,* a new species collected from decaying bark of *Quercus*, is described and illustrated. The fungus is characterized by its allantoid to fabiform, unicellular, smooth, brown conidia. Illustrations and a key to accepted species of *Craspedodidymum* are provided.

Key words—asexual fungi, neotropic, systematics

Introduction

Craspedodidymum Hol.-Jech. was introduced by Holubová-Jechová (1972) with *C. elatum* Hol.-Jech. as type species. Another 13 species were added by Lunghini & Onofri (1980), Rao & Hoog (1986), Subramanian & Bhat (1989), Mercado Sierra & Mena Portales (1992), Bhat & Kendrick (1993), Yanna & al. (2000), Pinruan & al. (2004), Ma & al. (2011) and Mel'nik & al. (2014). Subsequently, however, *C. hyalosporum* was recombined into the new genus *Anacraspedodidymum* (Silva & al. 2014). *Craspedodidymum* is distinguished by macronematous, unbranched or dichotomously branched, multiseptate, brown conidiophores with monophialidic integrated, determinate, terminal conidiogenous cells that may (but only rarely) extend sympodially. The conidiophores very often regenerate with several enteroblastic percurrent

extensions through the conidiogenous loci, which are flaring, funnel-shaped collarettes. The conidia are globose, ellipsoid, pyriform, obovoid or variably shaped, unicellular or septate, brown or dark brown, sometimes accumulating in dark pigmented masses.

During a mycological survey of microfungi associated with plant materials in a Guatemalan forest, a conspicuous fungus was collected that is described here as a new *Craspedodidymum* species.

Materials & methods

Individual collections were placed in plastic bags, taken to the laboratory, and treated according to Castañeda-Ruiz & al. (2016). Mounts were prepared in polyvinyl alcohol-glycerol (8 g PVA in 100 ml of water, plus 5 ml of glycerol) and lactofuchsin (0.1 g acid fuchsin, 100 ml 85% lactic acid, following Carmichael 1955) or in lactic acid (90%). Measurements were made at a magnification of ×1000 under a Leica DM750 microscope with bright field optics, and photomicrographs were taken using a Leica ICC50 E camera. The holotype was deposited in the Fungal Dried Reference Collection of the Universidad de San Carlos de Guatemala, Ciudad de Guatemala, Guatemala (MICG).

Taxonomy

Craspedodidymum guatemalense Figueroa, Bran, O. Morales & R.F. Castañeda, **sp. nov.** FIG. 1

INDEX FUNGORUM IF 554791

Differs from *Craspedodidymum siamense* by its allantoid to fabiform conidia.

TYPE: Guatemala, Senderos de Alux ecological park, San Lucas Sacatepéquez, Sacatepéquez, 14°36′N 90°38′W, on decaying bark of *Quercus* sp., 21.VII.2017, coll. R. Figueroa (**Holotype**, MICG 5551).

ETYMOLOGY: Latin, *guatemalense*, in reference to Guatemala.

COLONIES on the natural substratum effuse, hairy, brown. Mycelium superficial and immersed, composed of septate, branched, brown, smooth-walled hyphae, 2–3 µm diam. CONIDIOPHORES macronematous, mononematous, unbranched, erect, straight, with 1–7 enteroblastic percurrent extensions, cylindrical, 5–8-septate, brown below, pale brown toward the apex, smooth, 110–170 × 3.5–5 µm, CONIDIOGENOUS CELLS monophialidic, integrated, terminal, determinate or enteroblastic percurrent extended, pale brown, 8–16 × 4–5 µm, with a funnel-shaped collarette, 1.5–2.5 µm deep, 2–2.5 µm diam at the opening. CONIDIA solitary, acrogenous, allantoid to fabiform, unicellular, smooth-walled, brown, 8.5–12.5 × 4–5 µm, sometimes accumulating in brown to dark brown masses.

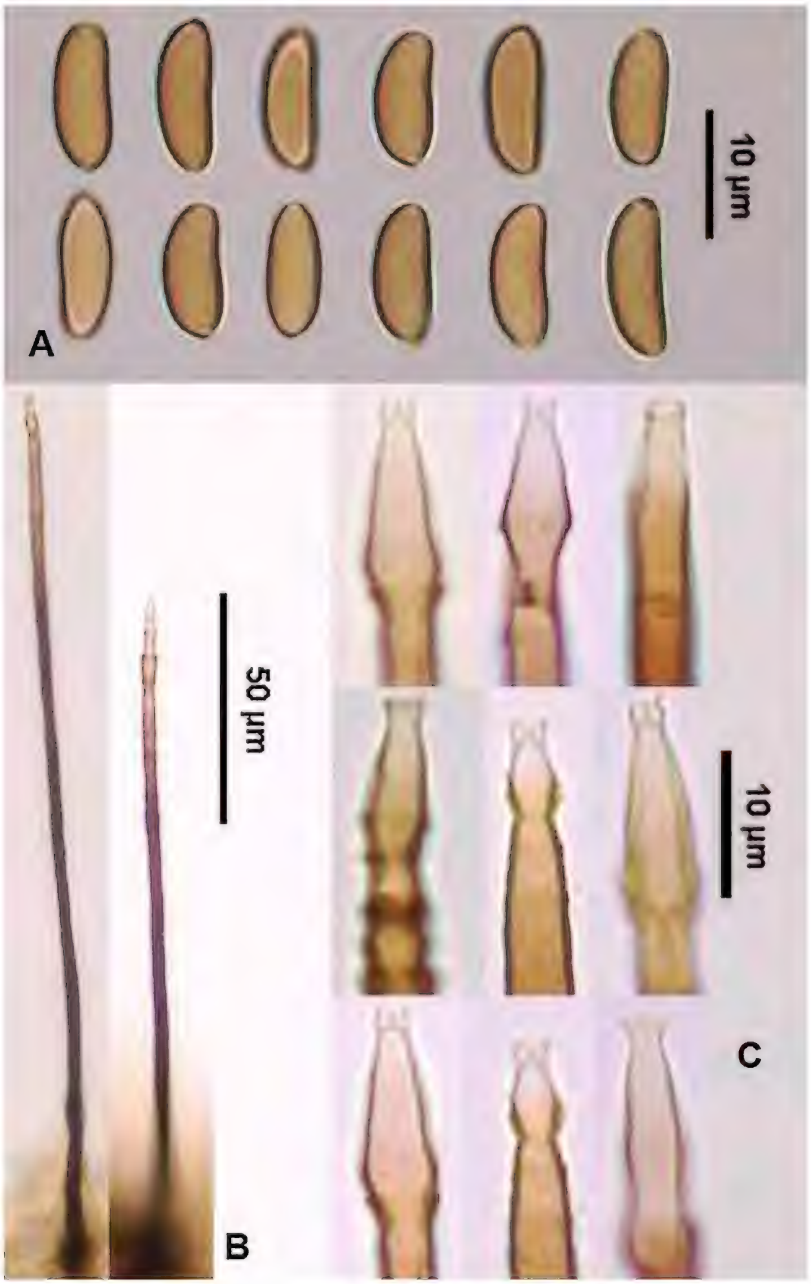

Fig. 1. *Craspedodidymum guatemalense* (ex holotype, MICG 5551).
A. Conidia; B. Conidiophores; C. Conidiogenous cells.

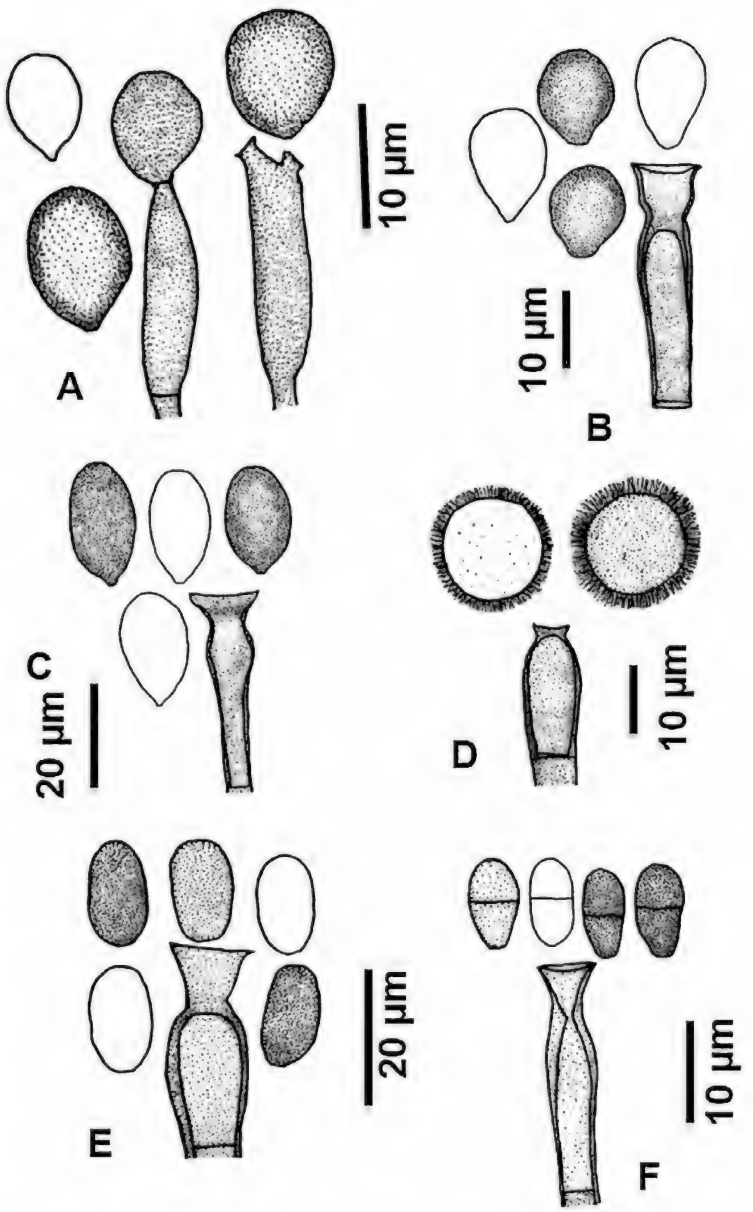

Fig. 2. *Craspedodidymum* spp., conidiogenous cells and conidia: A. ***C. abigianense*** (Lunghini & Onofri 1980); B. ***C. cubense*** (Mercado Sierra & Mena Portales 1992); C. ***C. elatum*** (Holubová-Jechová 1972); D. ***C. fimbriatum*** (Bhat & Kendrick 1993); E. ***C. fujianense*** (Ma & al. 2011); F. ***C. keniense*** (Kirk 1985, as *Dischloridium keniense*).

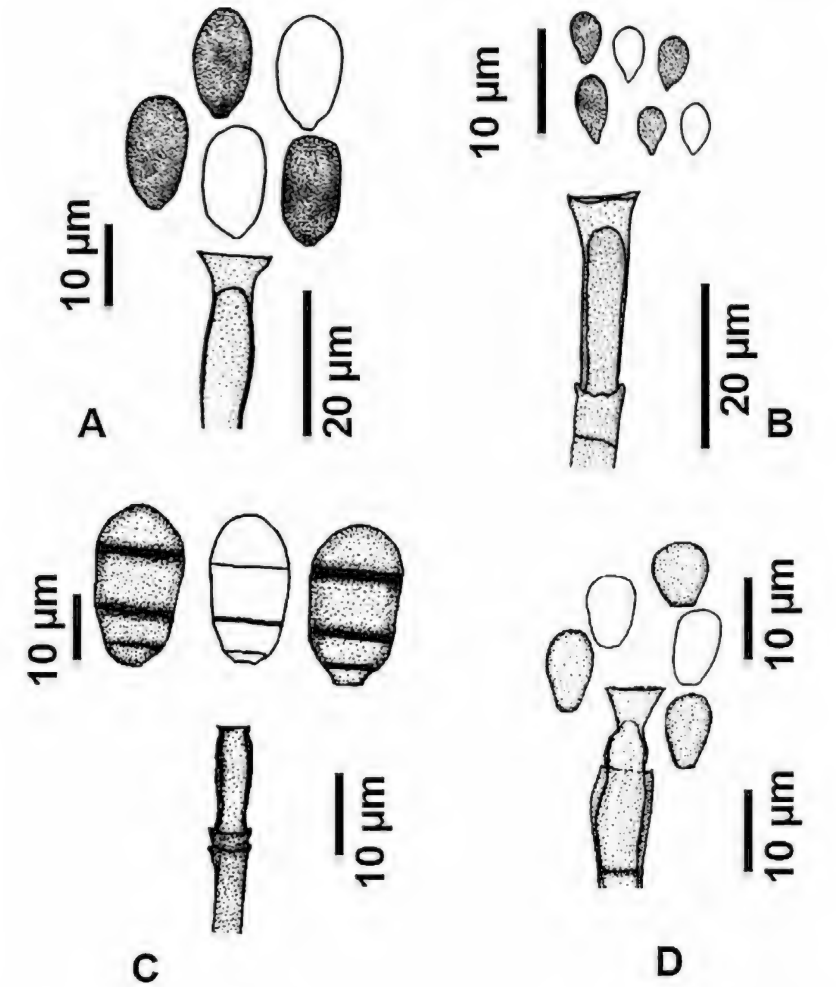

Fig. 3. *Craspedodidymum* spp., conidiogenous cells and conidia: A. ***C. licualae*** (Pinruan & al. 2004); B. ***C. microsporum*** (Pinruan & al. 2004); C. ***C. nigroseptatum*** (Yanna & al. 2000); D. *C. proliferans* (Rao & Hoog 1986).

Notes: There are 13 previously accepted species of *Craspedodidymum*: *C. abigianense*, *C. cubense, C. elatum*, *C. fimbriatum*, *C. fujianense*, *C. keniense*, *C. licualae, C. microsporum*, *C. nigroseptatum*, *C. proliferans*, *C. pulneyense*, *C. seifertii,* and *C. siamense* (Index Fungorum 2017) (Figs 2–3). Of these only *C. siamense* Pinruan is superficially similar to *C. guatemalense* in producing unicellular (not globose or near globose conidia); *C. siamense* differs in larger

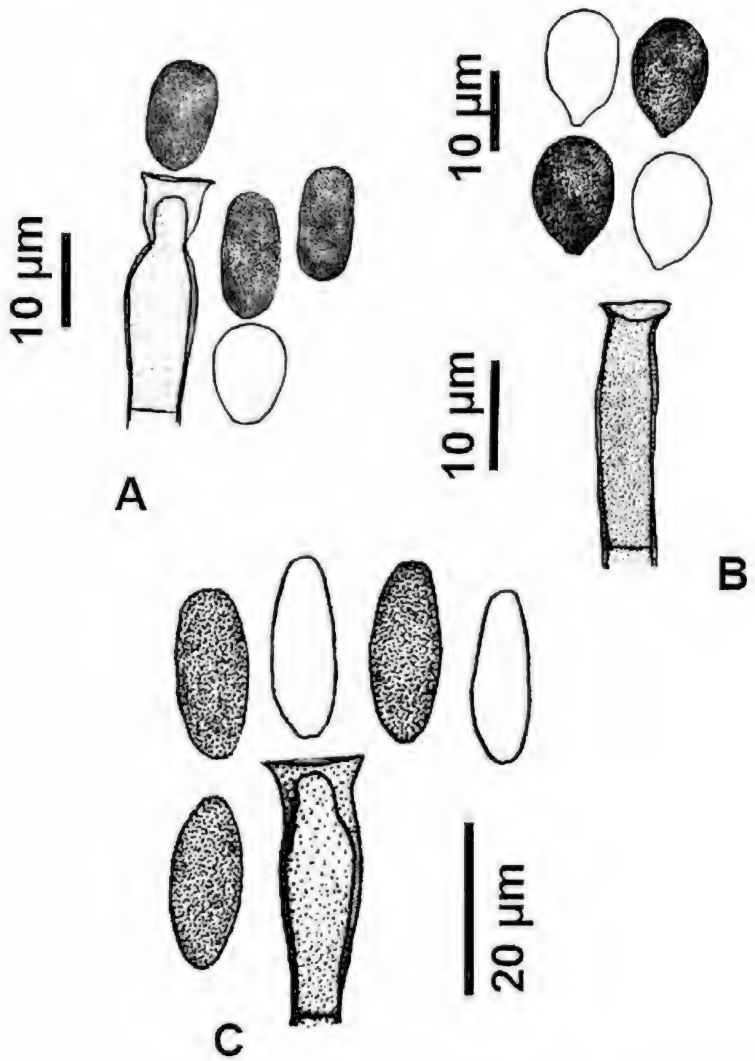

FIG. 4. *Craspedodidymum* spp., conidiogenous cells and conidia: A. ***C. pulneyense*** (Subramanian & Bhat 1989); B. ***C. seifertii*** Mel'nik & al. 2014); C. ***C. siamense*** (Pinruan & al. 2004).

funnel-shaped collarettes (12.5–15 µm diam at the opening) and ellipsoid to somewhat fusiform, medium brown, larger conidia (15–20 × 6.2–7.5 µm; Pinruan & al. 2004).

Key to accepted species of *Craspedodidymum*

1. Conidia unicellular 2
   Conidia septate 13
2. Conidiogenous cells mono- or rarely polyphialidic, conidia globose or obovoid, papillate at the base, brown, 13.5–14.5 × 14.5–16.5 µm ..... *C. abigianense*
   Conidiogenous cells always monophialidic 3
3. Conidia globose or subglobose to broadly obovoid 4
   Conidia not as above 7
4. Conidia fibrillose with numerous acellular curved appendages forming a pile or coat on the surface, mid brown, 18–24 µm diam ..... *C. fimbriatum*
   Conidia not fibrillose 5
5. Conidia ≤5 µm wide, papillate at the base, pale brown, 5–6.2 × 3.5–4 µm ..... *C. microsporum*
   Conidia >5 µm wide 6
6. Conidia solitary, sometimes pyriform, dark brown, 11.5–15 × 10.5–13 µm ..... *C. cubense*
   Conidia arranged in false chains, brown to dark brown, 18–22 × 13.2–19 µm ..... *C. seifertii*
7. Conidia obovoid to cuneiform 8
   Conidia not as above 9
8. Conidia not papillate, brown, 10–14 × 8–11 µm ..... *C. proliferans*
   Conidia papillate, brown, 13.7–17.5 × 7.5–10 µm ..... *C. licualae*
9. Conidia mostly oblong or cylindrical 10
   Conidia not as above 11
10. Conidia brown, 13–15.5 × 7.5–10 µm ..... *C. fujianense*
    Conidia brown, 11–17 × 7–8.5 µm ..... *C. pulneyense*
11. Conidia fabiform to allantoid, brown, 8.5–12.5 × 4–5 µm ..... *C. guatemalense*
    Conidia ellipsoid 12
12. Conidia papillate at the base, dark brown, 13.5–20 × 7.5–11.5 µm ..... *C. elatum*
    Conidia not papillate at the base, medium brown, 15–20 × 6.2–7.5 µm ..... *C. siamense*
13. Conidia 1-septate, broadly ellipsoid to obovate, truncate at the base, brown, flattened, 11–18 × 6–9 µm ..... *C. keniense*
    Conidia 3- (to 4-) septate, with two dark brown distal cells, broadly ellipsoidal to obovoid, truncate at the base, 24–30 × 13–19 µm ..... *C. nigroseptatum*

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Peltigera neodegenii sp. nov. from Central China

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Abstract—*Peltigera neodegenii* from Central China is described and illustrated as a new species. It is similar in general appearance to *P. degenii, P. membranacea,* and *P. canina*, but is distinguished by its shiny upper surface in the central part of lobes, tomenta around the margins of upper surface of lobes, distinct and raised veins on the lower surface of lobes, and simple rhizines. Comparisons of the ITS (ITS1, 5.8S, ITS2) sequences of the nuclear ribosomal DNA repeat tandem, both in phylogenetic analysis and secondary structure models of ITS2, support the taxonomic distinctness of this species.

Key words—biodiversity, cyanolichen, *Peltigeraceae*, *Peltigerales*, Shennongjia Mountain

Introduction

Peltigera Willd. (*Peltigeraceae*), characterized by large foliose thalli and a worldwide distribution in moist habitats, currently includes more than 90 species (Kirk & al. 2008; Han & al. 2013, 2015; Manoharan-Basil & al. 2016). In modern lichen taxonomy, molecular techniques have been used widely to delimit species within the genus (Goffinet & Miądlikowska 1999, Sérusiaux & al. 2009). The nrDNA ITS region, the main molecular marker or barcode used to characterize fungal and lichen species (Schoch & al. 2012, Guo 2013),

# Liu-Fu Han, Xiao-Min Xu & Jing-Yuan Yang contributed equally to this work.

has been helpful in revealing phylogenetic relationships in *Peltigera* (Goffinet & al. 2003; O'Brien & al. 2009; Sérusiaux & al. 2009; Han & al. 2013, 2015; Manoharan-Basil & al. 2016).

Peltigera has been relatively well studied in the northern hemisphere and a phylogeny combining morphological and chemical characters with LSU nrDNA sequence analysis has been carried out (Miądlikowska & Lutzoni 2000). In Asia, about 40 species have been reported, and the nrDNA ITS sequence data of most species are available from GenBank (Martínez & al. 2003; Han & al. 2013, 2015). Of the 30 species recorded in China (Chen 1986; Vitikainen 1986; Wei 1991; Stenroos & al. 1994; Wu & Liu 2012; Han & al. 2013, 2015; Niu & al. 2016), 14 belong to the *P. canina* group, in *P.* sect. *Peltigera*. During our ongoing study of *Peltigera* species in China, we identified a new species based on morphological characteristics and nrDNA ITS sequence data, which we describe here as *Peltigera neodegenii*. We also compare the secondary structure of the *P. neodegenii* nrDNA ITS2 region with those of two closely related species, *P. degenii* Gyeln. and *P. membranacea* (Ach.) Nyl.

Materials & methods

Specimens & morphology

Specimens were collected from Shennongjia forest region in Central China and all examined morphologically using a Motic SMZ-140 or Zeiss-Stereo Discovery-V12 dissecting microscope and Leica DM500 compound microscope. Asci and ascospores were observed in sections of apothecia cut by hand with razor and mounted in water after staining in 0.2% toluidine blue for about 15 min. Descriptions and terminology follow Vitikainen (2004). Thin layer chromatography (TLC) was performed on all specimens using solvent systems C and G according to Orange & al. (2010). The specimens are deposited both in the Herbarium Mycologicum Academiae Sinicae-Lichenes, Beijing, China (HMAS-L) and the Lichen Section of Botanical Herbarium, Hebei Normal University, Shijiazhuang, China (HBNU).

DNA extraction, PCR amplification, sequencing

The samples of lobe tips were cut for DNA extraction from five specimens (including the type and three other *P. neodegenii* specimens and one *Peltigera praetextata* specimen). DNA was extracted using the DNAsecure Plant DNA Kit following the manufacturer's protocol. The ITS region was amplified according to Han & al. (2013, 2015). The entire nrITS region (ITS1+5.8S+ITS2) was targeted for Polymerase Chain Reaction (PCR) using primers ITS1F and ITS4 (White & al. 1990) in a 25 µL volume containing 0.75 units of TransStart Taq Polymerase, 2.5 µL of ITS buffer, 0.5 µl of a 5 µM solution of the primers, 2 µL of 2.5 mM for each dNTP solution, and 1 µL of genomic DNA. Thermal cycling conditions were 95°C for 3 min initiation, followed by 35 cycles at 94°C for 30 s, 54°C for 30 s, and 72°C for 1 min

with a final extension at 72°C for 10 min. PCR products were screened on 1% agarose gels stained with ethidium bromide and sequenced by the Genewiz Inc. (Suzhou, Jiangsu, China).

Phylogenetic analysis & sequence comparisons

The entire ITS sequences of five samples of our examined specimens and the 24 representatives selected from GenBank were aligned manually by both ClustalW and Muscle implemented in MEGA version6 (Tamura & al. 2013). We excluded the 3′ end of the 18S gene and the 5′ end of the 26S gene from the analyses. Sequences from the new species were aligned with GenBank ITS sequences from taxa sharing the most similarity with them as determined from morphological characters, Blast results of sequence data, and selected references (e.g., Miądlikowska & Lutzoni 2000, Goffinet & al. 2003, Miądlikowska & al. 2003, Sérusiaux & al. 2009).

Ambiguously aligned regions sensu Lutzoni & al. (2000) were delimited manually and excluded. The final matrix of the remaining 520 characters submitted to TreeBase with accession number TB2:S15264 may be obtained from the corresponding authors.

The evolutionary history was inferred from 29 nucleotide sequences by using both the Maximum Likelihood method based on the GTR+Γ model in MEGA6 and Bayesian inference (Huelsenbeck & Ronquist 2001) based on GTR model with rates = Invgamma. The secondary structures of ITS2 sequences for the new species and its most closely related species were predicted by The ITS2 Database III (Koetschan & al. 2010) and illustrated with PseudoViewer3 (Byun & Han 2009).

Results

Phylogenetic analysis and secondary structure of ITS2

The newly obtained sequences were submitted to GenBank. The entire ITS region, which was successfully sequenced for five samples from the collections from Mt. Shennongjia, Central China, comprises 573–574 bp (ITS1 = 216–217 bp; 5.8S = 157 bp; and ITS2 = 200 bp). We included ITS sequences from our new species and the GenBank reference sequences in the phylogenetic analyses. The data matrix of the aligned 29 sequences comprised 520 characters, of which 468 (90%) were constant and 52 (10%) were parsimony informative.

The hypervariable region in the internal transcribed spacer 1 (ITS1-HR; Miadlikowska & al. 2003) was calculated, with about 23% variable sites compared with *P. degenii*, for 86 positions in total (not shown).

The alignment dataset was analyzed using MrBayes for Bayesian inference and MEGA6 for Maximum Likelihood; similar topologies were obtained. A ML tree with bootstrap values (1000 replicats) and Bayesian posterior probabilities (BPP) at branches is shown in Fig. 1. In the phylogenetic tree, the new species formed a clade with *P. membranacea*. The different samples of the new species formed a clade with 70% ML support and 0.97 of BPP.

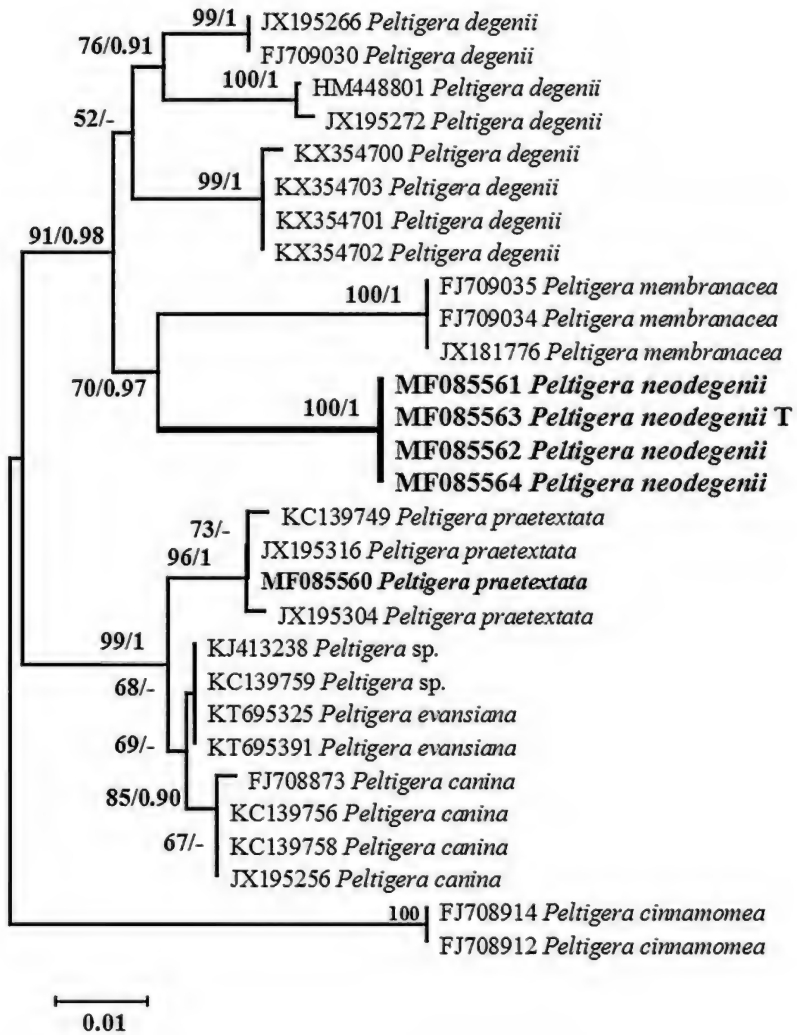

FIG. 1. Phylogenetic relationships among selected members (7 taxa, 29 sequences) closely related to *Peltigera neodegenii* based on ITS sequences. Values associated with internodes represent bootstrap support (ME-BP; before the slash) and posterior probability support (PP; after the slash). Only values ≥50% for ML-BP and ≥0·90 for PP are shown. *Peltigera cinnamomea* Goward was included as outgroup. Fonts in bold indicate newly generated sequences from specimens collected in Central China; the ex-type sequence of *P. neodegenii* (MF085563) is annotated: **T**. There was a total of 520 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 and MrBayes3.2.

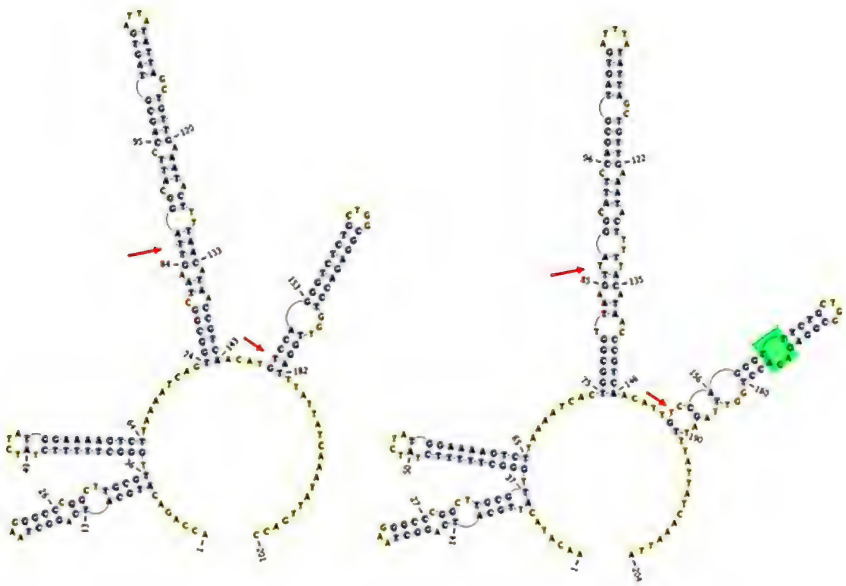

FIG. 2. The secondary structure model for the ITS2 sequence of *Peltigera neodegenii* (right, GenBank MF085563 *P. neodegenii* ex-type) and its template GI255347364 (left, GenBank FJ709030 *P. degenii*). Red arrows indicate a hemi-compensatory base change (hCBC) and the green box indicates a compensatory base change (CBC).

A high-quality secondary structure model of the ITS2 sequence representing the new species (GenBank MF085563) was derived from template GI255347364 (GenBank FJ709030, *Peltigera degenii*), the GenBank sequence closest to the new species, with 93% identity for ITS2 region (compared with only 90% identity between the new species and *P. membranacea*). The calculated free energy is –17.80 kcal/mol. Percentages of helix transfer are /100/100/96/85/ (threshold:75%; E-value: 1.2e-52) for four helices. The computed structure is shown in FIG. 2. One obvious difference between the structural models representing our new species and the closely related *P. degenii* includes one hCBC in the conserved part of helix III and one hCBC and two CBCs in the conserved part of helix IV.

Taxonomy

Peltigera neodegenii L.F. Han, S.Y. Guo & Xiao M. Xu, **sp. nov.** FIG. 3

MycoBank MB821289

Differs from *Peltigera degenii* by its upper surface that is shiny at the centre but tomentose towards the margin, and by the absence of marginal phyllidia.

TYPE: China, Hubei Province, Shennongjia, Jinhouling, 31°28′N 110°18′E, with mosses over rock and soil, 2600 m alt., 24 April 2014, Shou-Yu Guo, Liu-Fu Han 21112. (**Holotype**, HMAS-L; GenBank MF085563; **isotype**, HBNU).

ETYMOLOGY: Referring to the morphological similarity with *Peltigera degenii*.

THALLUS foliose, loosely appressed, medium to large, 8–15 cm in diam.; lobes thin, somewhat brittle, 8–20 mm wide, separate or sometimes loosely overlapping, short to elongate, irregularly branching; lobe tips rounded, predominantly downturned; LOBE MARGINS mostly even or weakly crisped, occasionally with lobules; UPPER SURFACE deep bluish green to blackish green (moist) or pale bluish grey to brown (dry), smooth and somewhat shiny centrally, white-gray tomentose near lobe tips, the tomentum becoming absent toward thallus centre, appressed to occasionally erect in part; soredia and isidia lacking, epruinose; LOWER SURFACE distinctly veined; veins usually whitish, not appearing to overlap, narrow, often raised, usually lacking tomentum, but sometimes distinctly erect-tomentose; interstices white, lenticular to polygonal, moderately deeply set; RHIZINES concolourous with veins to pale brown, irregular, discrete, threadlike, occasionally branched, 4.0–8.0 mm long, lacking tomentum or sometimes distinctly erect-tomentose; cortex c. 30–55 µm thick; photobiont layer 25–40 µm thick, containing *Nostoc*; MEDULLA white, c. 250–700 µm thick.

APOTHECIA growing at short lobe tips, erect, saddle-shaped, ≤5.0 mm in diam; apothecial margin smooth to crenulate; DISC red brown to dark brown, smooth; PARAPHYSES simple, septate, ± swollen at the apices and pigmented; ASCI clavate, *Peltigera*-type, colorless to pale, 8-spored; ASCOSPORES acicular, 3(–4)-septate, 60–95 × 2.5–5.5 µm. PYCNIDIA not seen.

SPOT TESTS: all negative.

SECONDARY METABOLITES: none detected.

ECOLOGY & DISTRIBUTION: On mosses and soil in subtropical mountain forest; known only from Shennongjia forest region, Hubei, China.

ADDITIONAL SPECIMENS EXAMINED:

Peltigera neodegenii: **CHINA**, **HUBEI PROVINCE**, **Shennongjia**, Jinhouling, 31°28′N 110°18′E, with mosses over rock and soil, 2600 m alt., 24 April 2014, Shou-Yu Guo, Liu-Fu Han 20973 (HMAS-L, GenBank MF085564); 21105 (HMAS-L, GenBank MF085561); 21111 (HMAS-L, GenBank MF085562).

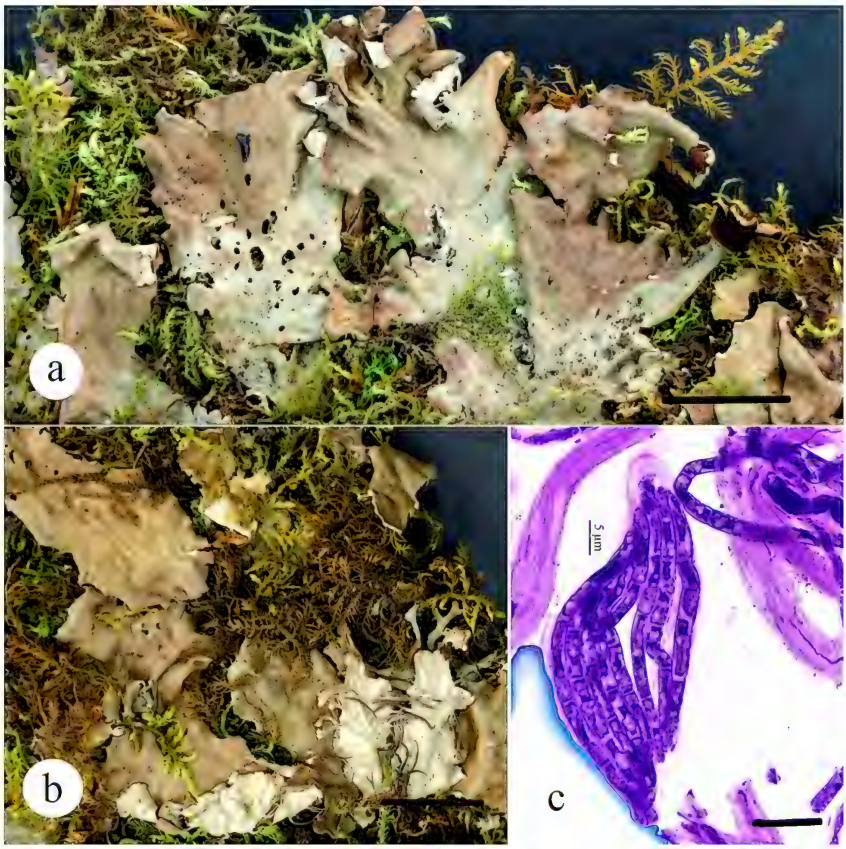

FIG. 3. *Peltigera neodegenii* (holotype, HMAS-L Guo & Han 21112). a: upper surface and apothecia (dry); b: upper surface, veins, and rhizines (dry); c: section through apothecium (stained by 0.2% toluidine blue for c. 15 min) showing asci and ascospores. Scale bars: a, b = 10 mm; c = 20 µm.

Peltigera praetextata (Flörke ex Sommerf.) Zopf: **CHINA, HUBEI PROVINCE, Shennongjia**, Laojunshan, 31°49′N 110°32′E, Shou-Yu Guo, Liu-Fu Han 20953 (HMAS-L, GenBank MF085560).

Discussion

The new species shows closer phylogenetic relationships with *Peltigera membranacea* and *P. degenii* than with other species in the ITS sequence analyses (FIG. 1), while the secondary structure models for ITS2 sequences suggest a greater with *P. degenii* (FIG. 2). (In contrast, there was very little similarity with the secondary structure model for the ITS2 sequence of *P. membranacea*, GenBank JX181776 [not shown].)

Morphologically *Peltigera neodegenii* is characterized by the presence of a cyanobacterial photobiont (*Nostoc* sp.) combined with a centrally shiny but marginally tomentose upper surface, predominantly downturned lobe tips, mostly even lobe margins, simple rhizines, and saddle-shaped apothecia. *Peltigera neodegenii* may be confused with *P. membranacea,* which also has a shiny central upper surface shiny and tomentose lobe ends, but which differs by sometimes wider (<4.0 cm) lobes, tomentose veins, and rope-like, tomentose rhizines (Wu & Liu 2012).

Peltigera canina (L.) Willd. and *P. degenii* also resemble *P. neodegenii* in morphology, but *P. canina* differs in its penicillate to confluent rhizines, while *P. degenii* differs in having glossy, glabrous upper surface and distinct marginal phyllidia (Goward & al. 1995; Wu & Liu 2012). *Peltigera evansiana* Gyeln. and *P. praetextata* are readily distinguished from *P. neodegenii: P. evansiana* by having granular isidia covering the lobe surfaces, and *P. praetextata* by having phyllidia produced at the margin and along cracks.

Previous studies that have used DNA sequence data for species recognition in *Peltigera* required monophyly both in single-locus ITS phylogenies and diagnosable morphological differences (Goffinet & Miądlikowska 1999; Goward & Goffinet 2000; Goffinet & al. 2003; Miądlikowska & al. 2003; Han & al. 2013, 2015). Here we add secondary structure models for ITS2 sequences, which are also useful characters to distinguish lichen species (Liu & Guo 2009, Cao & al. 2011, Guo 2013, Han & al. 2015). Coleman (2007) considered two organisms as representing different species if the conserved parts of their ITS2 sequences differed by more than one compensatory base change (CBC) or more than four hemi-compensatory base changes (hCBC). The threshold range between intraspecific and interspecific taxa for at least one CBC or four hCBCs in the four helices (I-IV) (Schultz & al. 2005, Guo 2013, Han & al. 2015). For *Peltigera neodegenii* and its template *P. degenii,* there are two hCBCs and two CBCs in the conserved part of helix III and helix IV, further supporting *P. neodegenii* as an independent species.

In conclusion, both distinctive morphological and molecular characteristics of *Peltigera neodegenii* confirm it as new to science.

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Arcyriatella congregata from Mexico: a second world record

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Abstract—The rare myxomycete *Arcyriatella congregata*, previously known only from Brazil, is redescribed from Mexican material. LM and SEM photographs of the most important micro- and macroscopic characters are provided.

Key words—chorology, *Mycetozoa*, myxobiota, Sinaloa, taxonomy

Introduction

Arcyriatella Hochg. & Gottsb. is a monotypic genus considered to be intermediate between *Arcyria* F.H. Wigg. and *Minakatella* G. Lister. It shares with *Arcyria* an evanescent peridium, which remains at the base of the pseudoaethalium, and a filamentous, reticulate capillitium. *Arcyriatella* is also close to *Minakatella* in its fructifications that assume the form of a pseudoaethalium, a strand-like hypothallus, and clustered spores (Hochgesand & Gottsberger 1989).

Arcyriatella congregata is known only from the state of Sao Paulo in Brazil, occurring on wood (Cavalcanti & al. 2014). Its taxonomic position is problematic, intermediate between the families *Trichiaceae* (Hochgesand & Gottsberger 1989, Keller & Braun 1999, Cavalcanti & al. 2014) and *Arcyriaceae* (Nannenga–Bremekamp 1991, Lado & Pando 1997, Neubert & al. 1993, Fiore-Donno & al. 2013 [as *Arcyriidae*]). We treat *Arcyriatella* as a member

of *Arcyriaceae*, pending molecular studies as proposed by Keller & Everhart (2008).

New material of *A. congregata* collected in the state of Sinoloa in Mexico provides additional information about this rare species and its distribution.

Materials & methods

Samples for light microscopy were mounted in Hoyer's medium. Macroscopic photographs were made with a Nikon SM2800 microscope, micrographs were obtained with a Nikon Eclipse 2000 microscope, and SEM micrographs were taken with a Zeiss DSM-950 microscope after critical point drying and sputtering, following the methods described by Moreno & al. (2015).

The specimens are stored in the Herbarium, Universidad de Alcalá, Alcalá de Henares, Spain (AH) and the Herbarium, Instituto de Ciencias Biomédicas, Juárez, Mexico (UACJ). Nomenclature follows Lado (2017).

Taxonomy

Arcyriatella congregata Hochg. & Gottsb.,
Nova Hedwigia 48: 486 (1989) Figs 1–11

Pseudoaethalium ferruginous brown to dark reddish brown, stalked, 1.5–2.5 × 0.8–1.5 × 1.5–2 mm, formed by tight fructifications, which are subcylindrical to subglobose, 0.1–0.3 mm diam., dark reddish brown, reminding one of a poorly developed *Stemonitis*. When mature they present a brownish to reddish brown capillitium, expanded in a very open net similar to *Arcyria*. Stalk membranous, expanded over the substrate or pendulate, light beige, being a continuation of the hypothallus (very similar to the condition found in *Minakatella longifila* G. Lister and *Badhamia utricularis* (Bull.) Berk.). Peridium membranous, evanescent, with metallic and iridescent tones, sometimes with portions remaining adhered to the stalk apex or to the fructification. Capillitium brownish, tubular, forming a net composed of filaments 4–6(–10) µm in diam. and meshes of very variable size, covered on the surface by abundant spines, internally reticulate to subreticulate as confirmed by SEM, with abundant nodes and broadened pyriform tips, subglobose, sometimes with a slight appendix. Spores dark brown in mass, clustered, easily separable, 8–10(–11) µm in diam., light brown, globose to subglobose, covered

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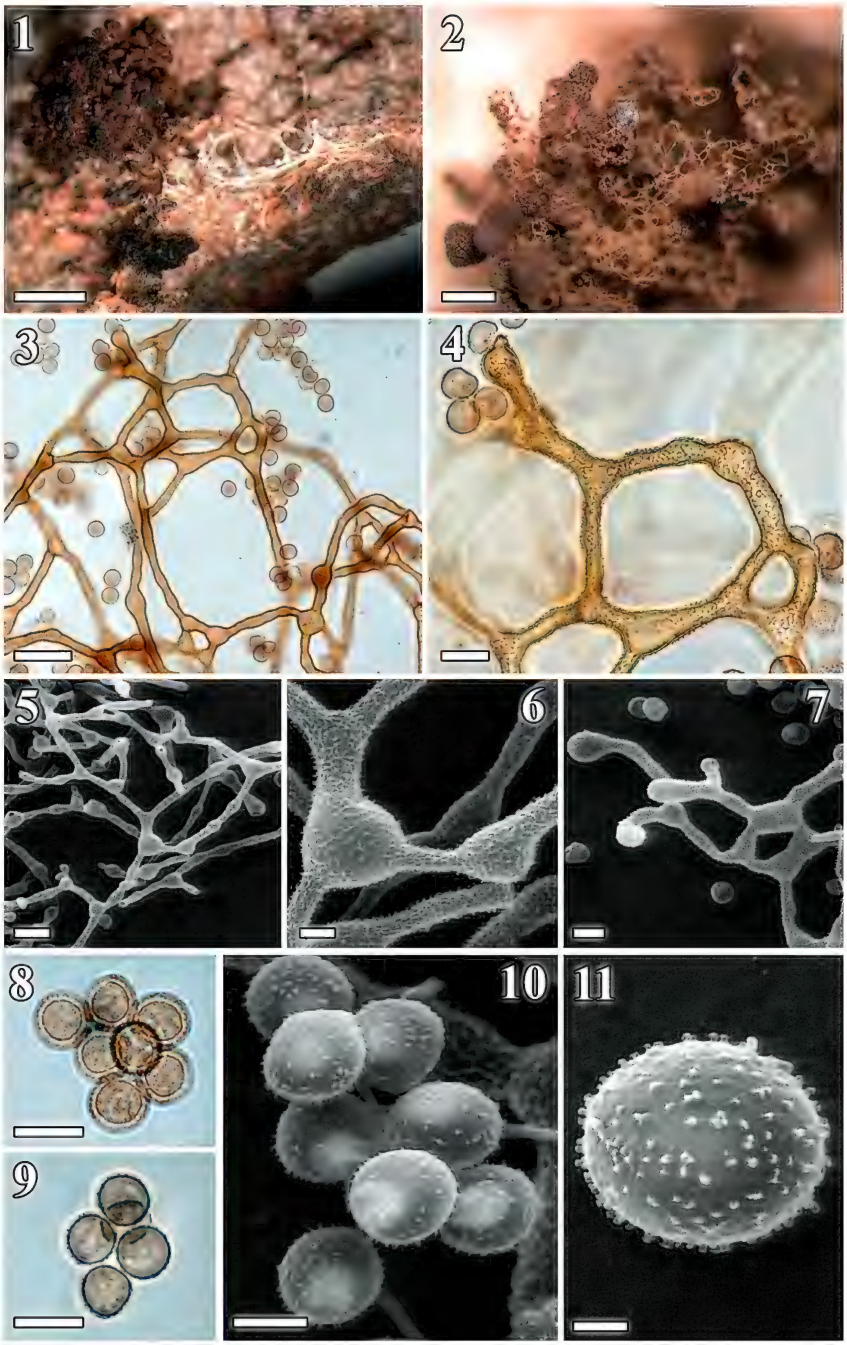
Figs 1–11: *Arcyriatella congregata*. (UACJ 3314): 1. Pseudoaethalium. (AH 46297): 2. Detail showing a pseudoaethalium with evanescent peridium; 3, 5. Capillitium; 4, 6. Reticulate capillitium with abundant nodes; 7. Detail of nodes and tips of the capillitium; 8, 9. Grouped spores (LM); 10. Grouped spores (SEM). 11. Detail showing spore ornamentation. Scale bars: 1 = 1 mm; 2 = 0.5 mm; 3 = 25 µm; 4, 7–9 = 10 µm; 5 = 20 µm; 6, 10 = 5 µm; 11 = 2 µm.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by dispersed warts. Under SEM the spore ornamentation is very loose and formed by small baculae of irregular distribution.

Material examined: **MEXICO. Sinaloa: Mazatlán**, El Quelite, bark of *Pithecellobium dulce* (Roxb.) Benth., 29.III.2016, leg. M. Lizárraga: put into moist chamber 1.VIII.2016, obtained 19.VIII.2016 (UACJ 3314); put into moist chamber 1.VIII.2016, obtained 25.VIII.2016 (AH 46297).

Observations — Our material differs from the specimens described by Hochgesand & Gottsberger (1989), because in our material the stalk is a continuation of the hypothallus, which extends over the substrate, very similar to the hypothallus of *Minakatella longifila* (Keller & al. 1973). Under the microscope, the grouped spores are easily separated; groups of spores are rare, and most of the spores are free. The spores are smaller than those in the type material (10–12 µm; Hochgesand & Gottsberger 1989). The reason for these differences may be explained by our harvesting material in moist chambers, where the organisms experience different developmental conditions than exist in the field. However, the fructifications in the form of pseudoaethalia, the stalk, the capillitium with nodes and typical tips, and the clustered spores define the specimens as *Arcyriatella congregata*.

Acknowledgements

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Notes on rust fungi in China 6. Distribution of *Puccinia punctiformis* and occurrence of its albino teliospores

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Abstract—A rust fungus producing systemic infections on *Cirsium arvense* in China was confirmed as *Puccinia punctiformis,* although previously in China this fungus had been treated as *P. calcitrapae* var. *centaureae*. Its morphology is described based on Chinese specimens and its wide distribution in China is clarified by herbarium specimens. In field observations in Jilin Province, albino teliospores were found. The identity of the albino spores with *P. punctiformis* was confirmed by molecular analyses.

Key words—abnormal spore, *Compositae, Pucciniomycetes,* taxonomy, *Uredinales*

Introduction

Puccinia punctiformis is a common rust fungus that has been reported on *Cirsium arvense* in many parts of the world (Wilson & Henderson 1966, Cummins 1978, Hiratsuka & al. 1992, Azbukina 2005, Termorshuizen & Swertz 2011). This fungus causes a systemic infection and produces spermogonia, aecia, uredinia, and telia on the single host, although aecia and uredinia are morphologically similar (Wilson & Henderson 1966, Cummins 1978, Hiratsuka & al. 1992). The fungus survives in rhizomes and produces a sweet odor during formation of spermogonia. However, its life cycle has not been investigated sufficiently (Wilson & Henderson

1966, French & Lightfield 1990, Wandeler & Bacher 2006, Termorshuizen & Swertz 2011). Because its host plant, *C. arvense,* is a noxious perennial weed the fungus has been investigated as a biological control agent (Thomas & al. 1994, Wandeler & Bacher 2006), although with little success (Termorshuizen & Swertz 2011).

In China, the rust on *C. arvense* has been treated as *Puccinia calcitrapae* var. *centaureae* (DC.) Cummins (Tai 1979, Wei & Wang 1986, Zhuang 2003). Recently, abundant infection of a rust fungus on *C. arvense* was observed in the campus of Jilin Agricultural University, Changchun, Jilin Province, China. Dark brown telia on the surface of plant leaves were frequently observed, but spermogonia and aecia/uredinia were rare. The rust on *C. arvense* was morphologically similar to *P. punctiformis* and, therefore, we carried out morphological observations to confirm its identity. We also borrowed rust specimens deposited as *P. calcitrapae* var. *centaureae* on *C. arvense* in HMAS Fungarium, Beijing, China. The results of our morphological observations of a rust fungus on *C. arvense* and its distribution in China are reported. During field surveys, a rust fungus on *C. arvense* with albino systemic telia was found on leaves of one plant in June 2017. Its identity with a rust fungus producing dark brown telia on *C. arvense* was confirmed by molecular analyses.

Materials & methods

Rust specimens on *Cirsium arvense* were collected in Changchun, Jilin Province, China, from 2015 to 2017 and used for morphological observations. These specimens were deposited in the Herbarium of Mycology, Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, China (HMJAU). Rust specimens on *C. arvense* were also borrowed from the Fungarium, Institute of Microbiology Chinese Academy of Sciences (HMAS), for comparative morphology.

Light (LM) and scanning electron (SEM) microscopy were used to examine morphological characters including the size and shape of sori and spores, following the method reported by Ji & al. (2017b).

The identities of the dark brown and albino teliospores on *C. arvense* collected in the campus of Jilin Agricultural University, were confirmed through molecular analyses as described by Ji & al. (2017a). Total genomic DNA was separately extracted from dark brown and albino teliospores, and rDNA-ITS region was amplified and sequenced. Sequence data were deposited in GenBank.

Fig. 1. *Puccinia punctiformis:* spermogonial and aecial stages. A, C. Systemic spermogonia produced over whole leaf surface of Cirsium arvense; B. Vertical section of a spermogonium; D. Aeciospores with brown walls; E. Spermogonia and uredinoid aecia on the lower leaf surface; F. Vertical section of a uredinoid aecium; G. Uredinoid aecium (SEM); H. Aeciospores with spines (SEM). Scale bars: B, F, G = 50 μm; D = 30 μm; H= 5 μm.

Results & discussion

Spermogonial, aecial, and telial stages of the rust on *C. arvense* collected in Jilin Province were identified as *P. punctiformis* based on the morphological similarity with the descriptions by Wilson & Henderson (1966), Cummins (1978), Hiratsuka (1980), Hiratsuka & al. (1992), Azbukina (2005), and Termorshuizen & Swertz (2011) (Figs 1, 2). These stages were systemically scattered over the entire leaf surface. Following the spermogonial stage, pedicellate spores were produced in aecia without a peridium or sori called uredinoid aecia (Figs 1E, F). However, uredinia produced by infection of aeciospores were not confirmed in the specimens because of the similarity of aecial and uredinial stages. Most specimens on the synonymous *Cephalonoplos segetum* [≡ *Carduus segetum*], *Cirsium setosum*, and *C. arvense* [≡ *Carduus arvensis*], deposited in HMAS under the name *P. calcitrapae* var. *centaureae,* were morphologically identified as *P. punctiformis* based on their telia covering most of the leaf surface and teliospores that were shorter than those of *P. calcitrapae* var. *centaureae* (33–42 × 20–24 µm; Cummins 1978, Hiratsuka 1980, Hiratsuka & al. 1992). These *P. punctiformis* specimens were collected from a broad area of China.

Albino teliospores found on leaves of a single *C. arvense* plant were completely hyaline, including the walls (Figs 3C, D). Around this plant many plants producing the normal dark brown systemic telia were also found. Teliospores in dark brown telia were brown to dark brown. Except for the wall color, telia and teliospores of the albino form were morphologically similar to the dark brown form. The teliospore surfaces in both forms were finely verrucose (Figs 2F, 3F), but there were fewer and smaller verrucae in the albino form. The rDNA ITS sequences obtained from the two forms showed 99.7% similarity, with a sequence difference of 2 bp among 586 bp (albino form: HMJAU8555, GenBank MF397930; dark brown form: HMJAU8556, GenBank MF397931). The albino fungus was therefore confirmed as *P. punctiformis.* There were no other sequence data of this rust in GenBank. The occurrence of albino spores has been reported in smut fungi (Fischer & Holton 1957, Thomas 1984) and mushrooms (Murakami & Takemaru 1990). Albino spores apparently are rarely found in rust fungi, although there are reports of albino aeciospores in *Peridermium* (Mielke & Peterson 1967, Christenson 1969) and albino urediniospores in *Puccinia graminis* f. *tritici* Erikss. & Henning (Newton & Johnson 1927). This is the first report of albino teliospores in a rust fungus. We suspect that the albino

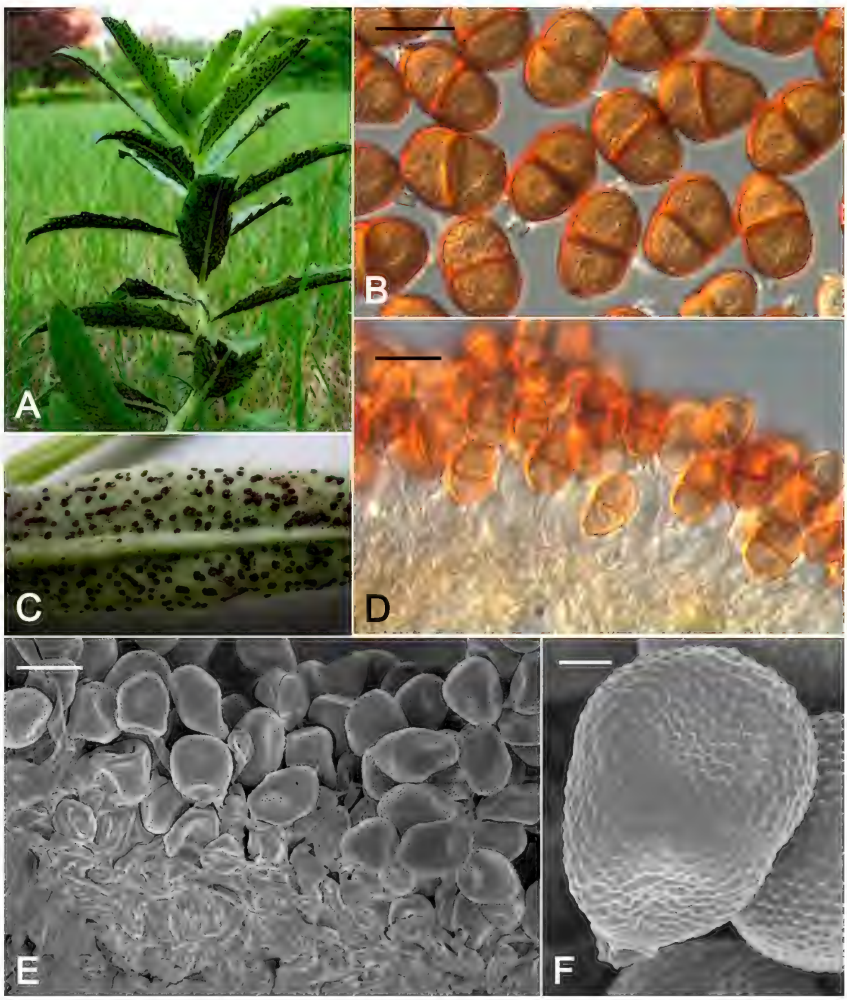

Fig. 2. *Puccinia punctiformis* telial stage. A, C. Systemic telia produced on the lower leaf surface of *Cirsium arvense;* B. Teliospores with dark brown walls; D. Vertical section of a telium; E. Telium (SEM); F. Teliospores with small verrucae (SEM). Scale bars: B, E = 30 μm; D = 40 μm; F = 5 μm.

form resulted from a mutation in a dark brown form, although further investigation is required. As neither teliospore form germinated after maturation, it is difficult to determine the functional differences between the two forms, including basidial formation and infection of plants.

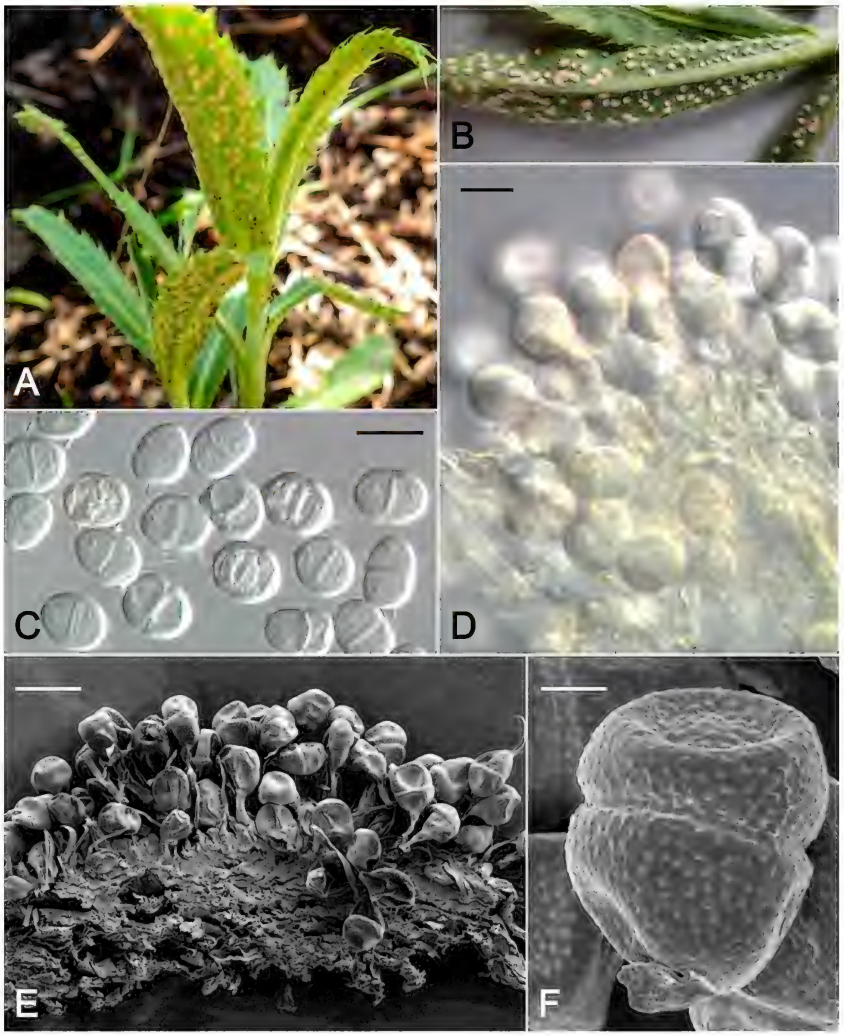

Fig. 3. *Puccinia punctiformis:* albino form. A, B. Systemic telia produced on the lower leaf surface of *Cirsium arvense;* C. Teliospores with hyaline walls; D. Vertical section of a telium; E. Telium (SEM); F: Teliospores with small verrucae (SEM). Scale bars: C, D = 35 µm; E = 30 µm; F = 5 µm.

Morphological features of this rust, including the albino form, and its distribution is provided in the following description, based on specimens collected in China.

Puccinia punctiformis (F. Strauss) Röhl.,
Deutschl. Fl., Ed. 2, 3(3): 131, 1813. FIGS 1–3

Spermogonia systemic with sweet odor, amphigenous, minute, scattered over whole leaf surface, orange-yellow, flask-shaped, type 4 of Cummins & Hiratsuka (2003). Aecia produced after spermogonia, systemic, amphigenous, pulverulent, brown, uredinoid, scattered over whole leaf surface. Aeciospores pedicellate, globoid, 18.5–25 × 16.5–23.5 µm (av. 22.5 × 20.5 µm); walls brown, mostly echinulate, 0.8–1.8 µm (av. 1.1 µm) thick; germ pores mostly 3, scattered. Uredinia and urediniospores could not be confirmed. Telia systemic, amphigenous, dark brown to black, scattered over whole leaf surface, first covered by epidermis, later pulverulent. Teliospores pedicellate, ellipsoid to broadly ellipsoid, 28–37.5 × 20–29 µm (av. 32.5 × 23.5 µm); walls brown, 1.5–3 µm (av. 2 µm) thick, verrucose with round and small verrucae; pedicels hyaline, deciduous, short. Albino telia white, similar to dark brown telia except in color. Albino teliospores pedicellate, ellipsoid to broadly ellipsoid, 27–38 × 16–28.5 µm (av. 32.5 × 24.5 µm); walls hyaline, 1–3 µm (av. 2 µm) thick, sparsely verrucose with minute verrucae; pedicels hyaline, deciduous, short.

SPECIMENS EXAMINED—On *Cirsium arvense* (L.) Scop.: **CHINA: JILIN PROVINCE**, 12 June 2015 (HMJAU8586); 3 September 2015 (HMJAU8587); 11 September 2015 (HMJAU8594); 12 June 2016 (HMJAU8593); 21 June 2016 (HMJAU8589); 24 June 2016 (HMJAU8592); 27 June 2016 (HMJAU8590); August 2016 (HMJAU8588); 21 May 2017 (albino telia: HMJAU855, GenBank MF397930; dark brown telia: HMJAU8556, GenBank MF397931); 4 September 2017 (HMJAU8591); 25 June 1950 (HMAS42950); **HENAN PROVINCE**, 13 June 1935 (HMAS08023); **JIANGSU PROVINCE**, April 1926 (HMAS12086); June 1932 (HMAS12023); 21 May 1929 (HMAS03343); 10 April 1924 (HMAS14498); 9 June 1932 (HMAS14381); 18 July 1930 (HMAS08025); **SHANDONG PROVINCE**, 13 June 1936 (HMAS14500); **SHANXI PROVINCE**, 30 June 1936 (HMAS14499); 23 August 1958 (HMAS36600); 26 May 1956 (HMAS36602); 21 July 1957 (HMAS36601); 16 September 1974 (HMAS36604); 5 May 1974 (HMAS36603); **SICHUAN PROVINCE**, 16 April 1938 (HMAS14497); 14 September 1989 (HMAS63912); **GUIZHOU PROVINCE**, April 1994 (HMAS73852); **GANSU PROVINCE**, 17 June 1943 (HMAS12022); 3 July 1958 (HMAS22374); **BEIJING MUNICIPALITY**, 5 November 1947 (HMAS12943); 1 August 1973 (HMAS36605); 27 May 1959 (HMAS49654); 16 June 2004 (HMAS172340, 172341); **LIAONING PROVINCE**, 13 July 1955 (HMAS17902); 2 May 1950 (HMAS22365); **XINJIANG PROVINCE**, 22 July 1959 (HMAS34490); 3 August 1977 (HMAS37762); 15 June 1959 (HMAS49655); **HEBEI PROVINCE**, 16 August 1964 (HMAS35363); 28 June 1964 (HMAS49658); **SHAANXI PROVINCE**, 26 June 1973 (HMAS54388); 15 June 1973 (HMAS54389); 4 April 1937 (HMAS22369); 28 April 1940 (HMAS22370); **TIBET PLATEAU**, 1 August 2012 (HMAS244560–244566).

HOSTS & DISTRIBUTION IN CHINA—On *Cirsium arvense* [= *Cephalonoplos segetum* (Bunge) Kitam.; = *Cirsium setosum* (Willd.) Besser ex M. Bieb.]: Beijing, Gansu,

Guizhou, Hebei, Henan, Jiangsu, Jilin, Liaoning, Shaanxi, Shandong, Shanxi, Sichuan, Tibet [Xizang], Xinjiang (Wei & Wang 1986, Zhuang 2003).

Puccinia punctiformis is widely distributed in China, where it was first recorded in 1924. This rust will presumably be found anywhere its host is present. However, in our Jilin Province field survey suggests that its occurrence and life cycle are affected by temperature and growing condition of its host.

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Rhexoampullifera marquesii sp. nov. on submerged twigs from a stream in Brazil

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Abstract—The new species *Rhexoampullifera marquesii*, collected in Bahia state, northeast Brazil on submerged decaying twigs, is described and illustrated. The fungus is mainly characterized by cylindrical, 6–15-septate, brown to dark brown conidia. A key and a synopsis table to *Rhexoampullifera* species are provided.

Key words—asexual fungi, taxonomy, tropical fungi, Brazilian Semi-arid region

Introduction

Rhexoampullifera P.M. Kirk is characterized by micronematous conidiophores and monoblastic or polyblastic conidiogenous cells producing septate conidia in simple or branched chains after rhexolytic secession (Kirk 1982). The conidial ontogeny observed in *Rhexoampullifera* is strongly related to thallic-arthric conidiogenesis, and rhexolytic disarticulation occurs in previously delimited cells of conidiogenous hyphae (Seifert & al. 2011). During a mycological survey of conidial fungi occurring on submerged decaying plant debris in the northeastern semi-arid region of Brazil, an interesting specimen was found that was clearly congeneric with *Rhexoampullifera* but differing from previously described species. We describe the fungus here as a new species.

Materials & method

Samples of submerged decaying plant material from Serra da Fumaça, Bahia, Brazil, were placed in plastic bags. The materials were washed in tap water and placed on

moist filter paper in 100 mm diam. glass Petri dishes. The dishes were then placed in plastic containers (150 L capacity), containing 200 mL sterile water and 2 mL glycerol, and incubated at room temperature (Castañeda-Ruiz & al. 2016). The plant material was regularly examined for the presence of microfungi during a month of incubation. Mounts were prepared in PVL (polyvinyl alcohol and lactic acid) and measurements were taken at ×1000. Microphotographs were made using a BX51 Olympus microscope equipped with bright field and Nomarski interference optics and a DP25 Olympus digital camera. The specimen was deposited in the Herbarium of Universidade Estadual de Feira de Santana, Bahia, Brazil (HUEFS).

Taxonomy

Rhexoampullifera marquesii L.B. Conc. & Gusmão, **sp. nov.** Fig. 1

MycoBank MB 824255

Differs from *Rhexoampullifera* spp. by its 6–15-septate conidia.

Type: Brazil. Bahia State: Pindobaçu, Serra da Fumaça, 10°39′S 40°22′W, on submerged decaying twig of unidentified plant, 27.IX.2016, coll. L.B. Conceição (**Holotype**: HUEFS 234852).

Etymology: The specific epithet is dedicated to the mycologist, Dr. Marcos Fabio Oliveira Marques (in memoriam), who also contributed to knowledge of hyphomycetes from the Brazilian Semi-arid.

Colonies on the natural substrate effuse, hairy, brown. Mycelium superficial, hyphae branched, cylindrical, septate, thick-walled, smooth, brown to dark brown, 5–6.3 µm diam.. Hyphopodia absent. Conidiophores micronematous or reduced to conidiogenous hyphae. Conidiogenous hyphae thallic-arthric, integrated or discrete, determinate, brown to pale brown. Conidial secession rhexolytic. Conidia catenate, cylindrical, dry, smooth, sometimes with slight constrictions, brown to dark brown; terminal conidia 58–93 × 4.3–6.3 µm, 6–15 septate with rounded apices; intercalary conidia 60–95 × 4.3–6.3 µm, 7–13 septate, truncate at the ends; separated by 2–4 µm long light brown cells with basal and apical frills formed by disarticulation of the apical and lateral branches of the conidiogenous hyphae.

Note: Previously, *Rhexoampullifera* comprised three species—the type species *R. fagi* (M.B. Ellis) P.M. Kirk & C.M. Kirk, found on dead *Fagus* leaves in United Kingdom; *R. moravica* Koukol, found on a fallen trunk in Czech Republic; and *R. subglobosa* R.F. Castañeda & al., found on leaves in Brazil (Castañeda-Ruiz & al. 2001, Ellis 1976, Kirk 1982, Koukol 2012).

The conidial ontogeny described and illustrated for the current *Rhexoampullifera* species is controversial. In *R. fagi* and *R. marquesii*, the conidial ontogeny is clearly thallic-arthric, with chains of conidia originating

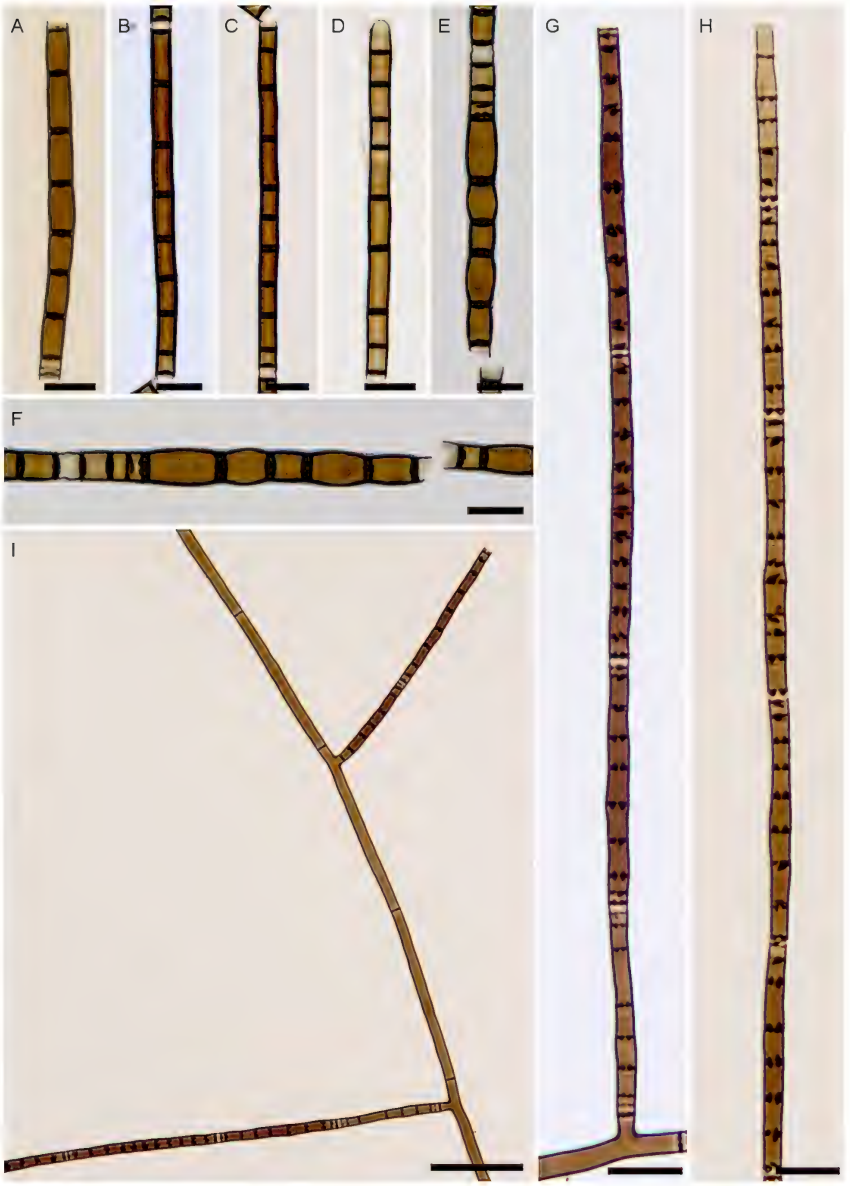

Fig. 1. *Rhexoampullifera marquesii* (holotype, HUEFS 234852). A–E. Conidia; F. Rhexolytic secession detail; G, H. Conidial chains; I. General aspect. Scale bars: A–F = 10 µm; G, H = 20 µm; I = 50 µm.

from a conidiogenous hypha and growing acropetally, but maturing basipetally, with the oldest conidia at the apical portion of the chains. However, a careful analysis of the description and illustration of *R. moravica* confirms that the conidia illustrated are clearly maturing acropetally, with the oldest conidia at the base (Koukol 2012: figs 1a, 2b). This same pattern can also be observed in the illustration of *R. subglobosa* (Castañeda-Ruiz & al. 2001: figs 1, 5), where the youngest conidia are at the apex.

Koukol's (2012) suggestion that in *R. moravica,* seceding conidia are not separated by a single separating cell but unequally on one side of a septum delimiting the two conidia suggests a secession intermediate between rhexolytic and schizolytic. *Rhexoampullifera marquesii,* which is congeneric with *R. fagi* based on conidiogenesis and conidial shape, differs in size and number of septa (Table 1) and absence of hyphopodia.

Future careful analysis of the types or access to the genetic material of these species will enable clarification of the phylogenetic relationships, but our observations indicate that *R. moravica* and *R. subglobosa* may not be congeneric with *Rhexoampullifera.*

Key to *Rhexoampullifera* species

1. Hyphae with hyphopodia ..... *R. fagi*
   Hyphae without hyphopodia 2
2. Conidia with fewer than 6 septa 3
   Conidia with more than 6 septa ..... *R. marquesii*
3. Conidia subglobose to turbinate, 0–2-septate ..... *R. subglobosa*
   Conidia cylindrical to slightly bent, 1–6-septate ..... *R. moravica*

Table 1. *Rhexoampullifera* conidial morphology

Species	Size (µm)	Shape	Septa	Reference
	Terminal / intercalary			
R. fagi	24–36 × 7–10 / 15–30 × 6–10	Cylindrical to doliiform	4 / 1–2	Kirk (1982)
R. marquesii	58–93 × 4.3–6.3 / 60–95 × 4.3–6.3	Cylindrical	6–15 / 7–13	This paper
R. moravica	23–36(–42) × 4–5 / 27–40(–45) × 4.5–6.5	Narrowly cylindrical to slightly bent	1–5 / (0)2–6	Koukol (2012)
R. subglobosa	12–24 × 10–13 / 19–21 × 9–14	Subglobose to turbinate / Cylindrical to navicular	0–2 / 0–2	Castañeda-Ruiz & al. (2001)

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Leucoagaricus pabbiensis sp. nov. from Punjab, Pakistan

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Abstract—A novel species, *Leucoagaricus pabbiensis*, is described and illustrated from Pabbi Forest Park, Punjab, Pakistan. The new species belongs to *Leucoagaricus* sect. *Leucoagaricus* and is characterized by a dark reddish brown central disc on a milky white pileus with radial reddish orange fibrils towards the margin. The creamy stipe, which is longer than the pileus is wide, has an ascending annulus; the basidiospores are lacrymoid to amygdaliform and dextrinoid; cheilocystidia are clavate to narrowly clavate; and both stipitipellis and pileipellis are made up of septate hyphae arranged as a cutis. The new agaric closely resembles *L. badius* but the novelty of *L. pabbiensis* is confirmed by morphological and molecular characters.

Key words—*Agaricaceae*, Gujrat, ITS region, macrofungi, Pakistan

Introduction

Salient characters of the genus *Leucoagaricus* Locq. ex Singer (*Agaricaceae*) are a fibrillose to squamulose pileus surface, white to cream or pink spore print, non-striate pileus margin, free lamellae, dextrinoid spores that are metachromatic in cresyl blue, and hyphae lacking clamp connections (Singer 1986, Vellinga 2001). *Leucoagaricus* is one of the basidiomycete genera with an extensive geographical distribution. More than 100 species are distributed from temperate to tropical regions of the world. Recent reports of new *Leucoagaricus* species throughout both northern and southern hemispheres suggest that many species of *Leucoagaricus* are still undescribed (Kumar & Manimohan 2009, Vellinga 2010, Vellinga & al. 2010, Liang & al. 2010, Vellinga & Balsley 2010,

Ge 2010, Muñoz & al. 2012, 2014, Malysheva & al. 2013, Kumari & Atri 2013, Ye & al. 2014, Ge & al. 2015, Qasim & al. 2015, Dovana & al. 2017, Hussain & al. 2018).

Leucoagaricus is poorly studied in Pakistan, and so far only nine species have been reported: three from Khyber Pakhtunkhwa province—*L. badius* S. Hussain & al, *L. pakistanensis* Jabeen & Khalid, *L. sultanii* S. Hussain & al.—and six species from lower elevations in district Lahore, Punjab province—*L. asiaticus* Qasim & al., *L. lahorensis* Qasim & al., *L. lahorensiformis* S. Hussain & al., *L. leucothites* (Vittad.) Wasser, *L. serenus* (Fr.) Bon & Boiffard, and *L. umbonatus* S. Hussain & al. (Ahmad & al. 1997, Qasim & al. 2015, Ge & al. 2015, Hussain & al. 2018).

Pabbi Forest Park (32°49′53″N 73°50′16″E) is part of the Himalayan foothills located in Gujrat district (Khan & al. 2016). The entire park covers about 160 km^2 over altitudes ranging from 280 to 410 m. Its rangeland vegetation type includes *Acacia modesta*, *Grewia tenax*, and *Prosopis juliflora* in the upper storey accompanied by the grasses *Cenchrus ciliaris*, *Cymbopogon jwarancusa*, *Cynodon dactylon*, and *Heteropogon contortus* (Arshadullah & al. 2007). Mean annual rain fall is 774 mm, concentrated mainly in July and August, and daily temperatures average 12°C in January and 33°C in June (Haider & al. 2011). Pabbi Forest Park was visited many times during the 2016–17 rainy season as part of a wider survey of the macrofungi of Pakistan. During these visits, two unknown *Leucoagaricus* specimens were collected. Preliminary morphological analysis indicating that the two specimens are conspecific was confirmed by ITS sequence analyses; they are proposed here as a new species, *L. pabbiensis*.

Material & methods

Morphology

Fresh basidiomata were described macroscopically and photographed with a Nikon D70 camera fitted with a Nikkor macro 18–77 mm lens. The specimens were dried under fan heater. Voucher specimens were deposited in the Herbarium, Department of Botany, University of the Punjab, Quaid-e-Azam Campus, Lahore, Punjab, Pakistan (LAH). Description terminology follows Vellinga (2001) and colors were coded according to Munsell (1994). The micromorphological descriptions are based on dried material rehydrated in 2% KOH. Melzer's reagent was used for amyloidity, and Congo red was used for staining cell walls of basidiomatal elements. Observations and measurements were recorded with the help of trinocular Labomed CXRII microscope at 4×, 10×, 40×, and 100× (oil immersion). The abbreviation [n/m/p] indicates n basidiospores measured from m fruit bodies of p collections. At least 20 basidiospores plus 10 each of basidia, cystidia, and pileipellis and stipitipellis elements were measured from one

Fig. 1. *Leucoagaricus pabbiensis* (holotype, LAH35302): basidiomata.

specimen. Basidiospore dimensions were recorded as (a–)b–c(–d), where a = extreme minimum value, range b–c contains at least of 90% of the calculated values, and d = extreme maximum value; Q indicates individual spore l/w ratios and Q_{av} presents the average Q of all spores. Plant names were retrieved from the International Plant Name Index (http://www.ipni.org) and fungal names were retrieved from Index Fungorum (http://www.indexfungorum.org).

DNA extraction, PCR amplification, & DNA sequencing

DNA was extracted following the CTAB method (Bruns 1995). Universal primers ITS1 and ITS4 were used to amplify the rDNA Internal Transcribed Spacer region (ITS). PCR conditions followed Gardes & Bruns (1993). Forward and reverse sequences were obtained from BGI (China) also using the ITS1/ITS4 primer pairs, with a consensus alignment of those sequences reached using BioEdit ver. 7.2.5 (Hall 1999). Nucleotide sequences were compared by using BLAST at NCBI (https://www.ncbi.nlm.nih.gov/guide/). Multiple sequences were aligned online using MUSCLE (https://www.ebi.ac.uk/Tools/msa/muscle/). Sequences with >83% identity (and E = 0) and recently deposited sequences from species of *Leucoagaricus* sect. *Leucoagaricus* (formerly *L.* sect. *Rubrotincti;* Redhead 2016) from Pakistan and other parts of Asia were included in the final data set to reconstruct the phylogeny. The phylogram, generated in RAxML-HPC2 using XSEDE tool (8.2.10), presents 39. sequences of *Leucoagaricus* plus one sequence of *Cystolepiota seminuda* (Lasch) Bon as outgroup.

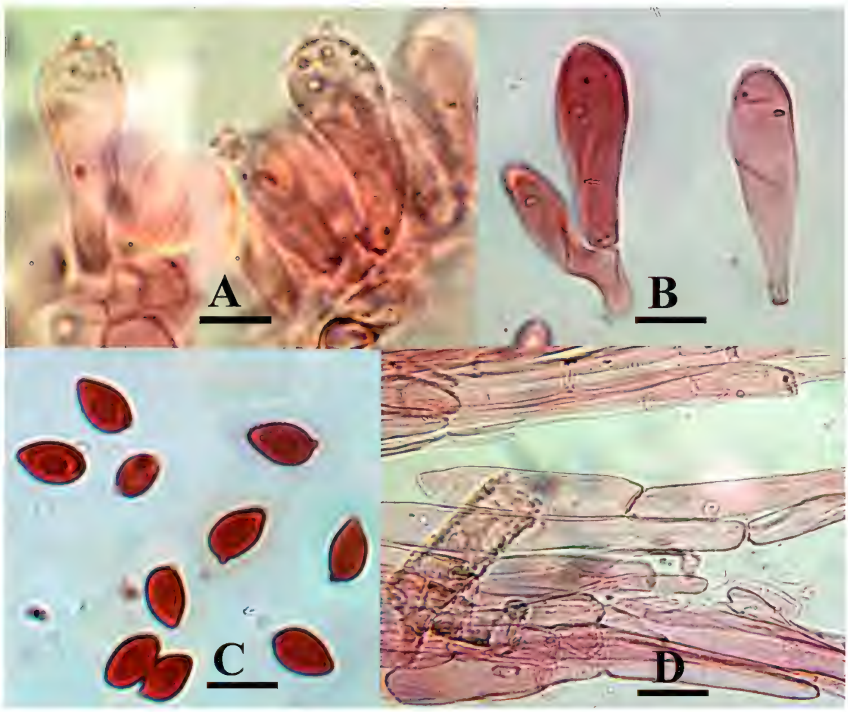

Fig. 2. *Leucoagaricus pabbiensis* (holotype, LAH35302): A. basidia; B. cheilocystidia; C. basidiospores; D. stipitipellis. (All mounts prepared in Congo red.) Scale bars: A = 10 µm, B = 9.5 µm, C = 8.3 µm, D = 7.2 µm.

Taxonomy

Leucoagaricus pabbiensis Usman & Khalid, **sp. nov.** Figs 1–3

MycoBank 824295

Differs from *Leucoagaricus badius* by its milky white pileus with dark reddish brown central disk and radially arranged reddish to orange fibrils, and its larger basidia and basidiospores.

Type: Pakistan. Punjab province, Pabbi Forest Park, 286 m a.s.l, on soil and leaf litter under *Acacia modesta* Wall., 11 August 2016, Muhammad Usman & Abdul Nasir Khalid MU02 (**holotype,** LAH35302: GenBank MG973423).

Etymology: The specific epithet "*pabbiensis*" (Latin) refers to the name of Pabbi Forest Park, the type locality.

Pileus 25–50 mm, when young convex, expanding to plane when mature, milky white with dark reddish brown (7.5R3/6) central disk and radially

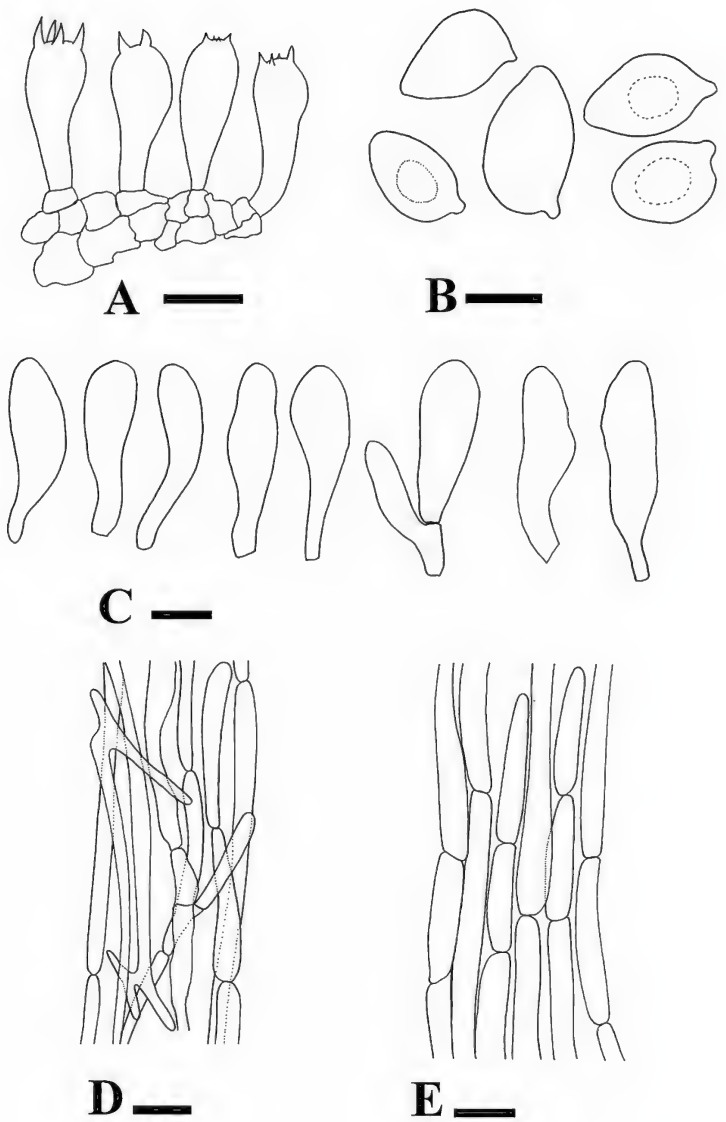

Fig. 3. *Leucoagaricus pabbiensis* (holotype, LAH35302): A. basidia; B. basidiospores; C. cheilocystidia; D. pileipellis; E. stipitipellis. Scale bar A = 16.5 µm, B = 4.5 µm, C = 10 µm, D = 10 µm, E= 12 µm. (Drawings by Muhammad Usman.)

arranged orangish yellow fibrils, orange brown (7.5R5/10) to light orange (7.5R5/16) scales radially arranged, fibrils uplifted; margins eroded. Lamellae free, milky white, crowded, thick, with eroded edge; short lamellulae present in series of 1–3. Stipe 25–60 × 3–5 mm, cylindrical, with subbulbous base, ≤10 mm in diameter, creamy white, smooth, sometimes with some very light pink brownish fibrils, hollow, with basal mycelial pad. Annulus thick, ascending, superior, persistent, creamy white; volva absent. Taste and aroma not recorded.

Basidiospores [50/3/2] (6.7–)7.7–9.6(–10.6) × (4.2–)4.7–5.5(–5.9) µm, on average 8.7 × 5.1 µm, Q = (1.32–)1.5–1.9(–2.2), Q_{av} = 1.7, hyaline in 2% KOH, dextrinoid, lacrymoid and amygdaliform in side-view, guttulate, smooth, thin-walled, apiculate. Basidia 20–32.4 × 8.5–11.4 µm, clavate, 2–4-spored, smooth with 2–4 prominent sterigmata. Cheilocystidia 28.3–36.2 × 8.6–12.3 µm, hyaline in 2% KOH, thin-walled, clavate to narrowly clavate, smooth. Pleurocystidia absent. Pileipellis a cutis, made up of 3.1–9.1 µm wide, septate, thin-walled hyphae, hyaline in 2% KOH; clamp connections absent. Stipitipellis made up of septate hyphae 4.8–8.9 µm in diameter, hyaline in 2% KOH, thin-walled, parallel in arrangement. Clamp connections absent.

Ecology & distribution—Solitary to gregarious on moist soil, rich in leaf litter under trees of *Acacia modesta*, fruiting during the monsoon season.

Additional specimen examined—**PAKISTAN. Punjab province**, Pabbi Forest Park, 286 m a.s.l, 20 August 2017, Muhammad Usman & Abdul Hameed MU78 (LAH35303: GenBank MG973424).

Phylogenetic analysis

Fig. 4

The phylogenetic tree comprises 40 sequences, of which 39 represent the ingroup, *L.* sect. *Leucoagaricus*. The final data set comprised 713 characters, of which 425 were conserved, 279 were variable, 188 were parsimony informative, and 91 were singletons.

The two *L. pabbiensis* sequences MG973423 and MG973424 were 100% identical, indicating that they are conspecific. In BLAST, the *L. pabbiensis* sequences showed maximum identity to a sequence named *Leucoagaricus* sp. from India (KR154966); and in the phylogenetic tree, these three sequences formed a clade with 94% bootstrap support (Fig. 4). *Leucoagaricus pabbiensis* also showed 99% similarity in BLAST with *L. badius* from Pakistan (KU647734–KU647736), but our phylogenetic analysis placed these two species in sister clades. *Leucoagaricus pakistanensis* (KU647726–KU647728), which is 91% identical to *L. pabbiensis,* forms a separate clade. Other *Leucoagaricus* species reported from Pakistan are *L. lahorensis* (KJ701794, KJ701796) 87% identical

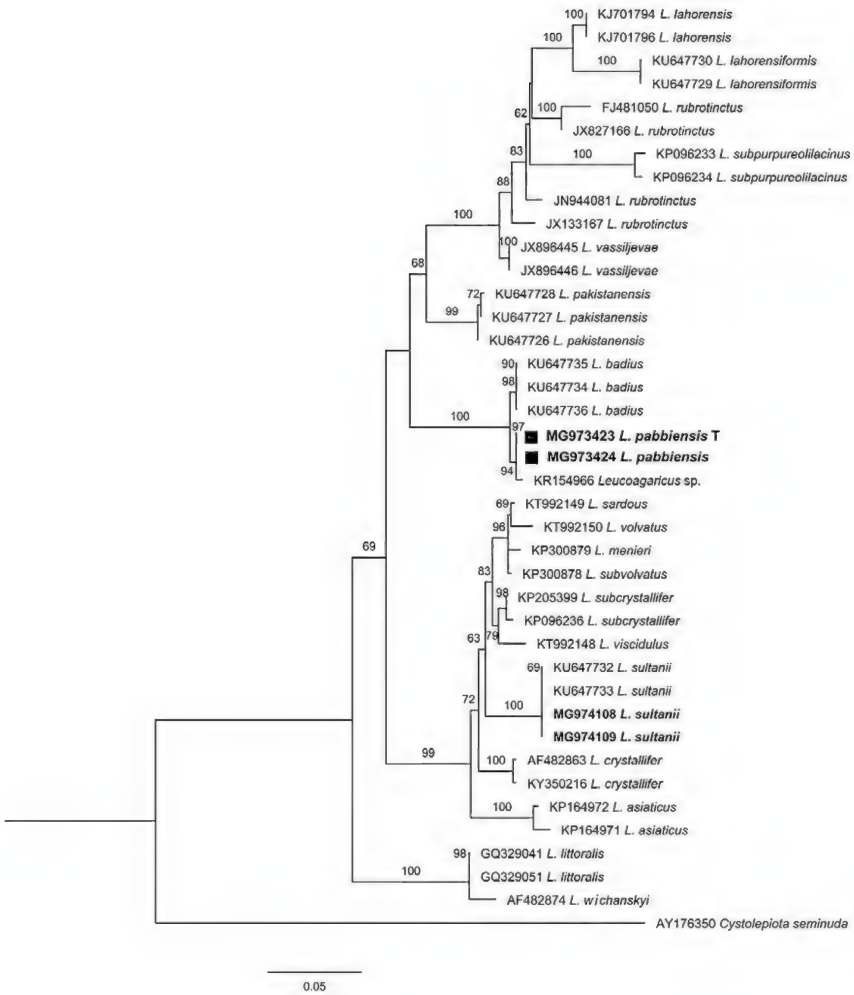

FIG. 4. Phylogram obtained from ITS sequence alignment of species of *Leucoagaricus* sect. *Leucoagaricus*. Bootstrap values >67% shown at nodes. *Leucoagaricus pabbiensis* sequences are indicated by ▪; and the type sequence is annotated **T**.

with *L. pabbiensis; L. lahorensiformis* (KU647729, KU647730) 87% identical; and *L. sultanii* (MG974108, MG974109, KU647732, KU647733) 90% identical. *Leucoagaricus rubrotinctus* (Peck) Singer (JN944081) from North America is 89% identical, and *L. subpurpureolilacinus* Z.W. Ge & Zhu L. Yang (KP096233, KP096234) from China is 87% identical.

Discussion

Leucoagaricus pabbiensis is characterized morphologically by a milky white pileus with dark reddish brown central disk and reddish to orange fibrils radiating towards the margin, lacrymoid to amygdaliform basidiospores, a cylindrical stipe with light pink fibrils and an ascending annulus. This set of morphological characters and phylogenetic analysis distinguishes *L. pabbiensis* from other closely related species in *L.* sect. *Leucoagaricus* from Pakistan and other parts of the world.

Leucoagaricus badius, a similar species from Malakand, Khyber Pakhtunkhwa, Pakistan, differs from *L. pabbiensis* by its white pileus with medium to dark red fibrillose squamules and deep red umbo, and its smaller basidiospores (6.5–7.5 × 4–5 µm) and basidia (14–17 × 6–8 µm; Hussain & al. 2018).

Another closely related species, *L. pakistanensis,* differs from *L. pabbiensis* by the color of its pileus and its smaller cheilocystidia (15–20 µm long; Hussain & al. 2018). *Leucoagaricus lahorensis* morphologically resembles *L. pabbiensis* only in the reddish color of its pileus and differs by its ellipsoid to broadly ellipsoid, larger basidiospores (8–10.6 × 6.4–7.6 µm; Qasim & al. 2015); our phylogenetic analysis (Fig. 4) also supports *L. pabbiensis* as distinct from *L. lahorensis. Leucoagaricus lahorensiformis* also shares some similarities with *L. pabbiensis* but differs by its smaller pileus (10–30 mm), basidiospores (6.5–7.5 × 3.5–4 µm), and basidia (18–22 × 6–9 µm) (Hussain & al. 2018).

Leucoagaricus pabbiensis also resembles *L. rubrotinctus,* the type species of the genus (Redhead 2016), first described from North America; but in *L. rubrotinctus* the whole pileus is orange-red and covered by orange fibrils, without showing the white context (Peck 1884, as *Agaricus rubrotinctus* Peck, nom illeg.). Phylogenetically these two species form separate lineages.

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Regional annotated mycobiota new to the Mycotaxon website

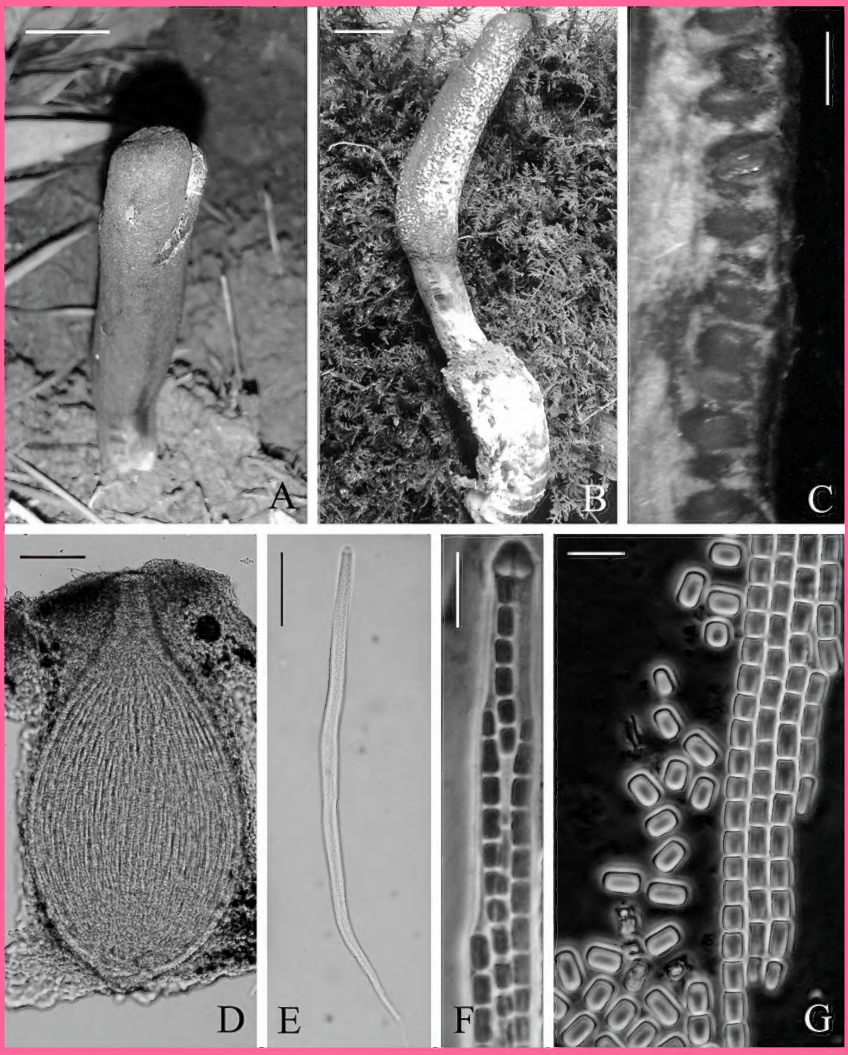
Abstract—Mycotaxon is pleased to add to our 'web-list' page the following new annotated species distribution list under Central America (Costa Rica): A check list of asexual fungi from Costa Rica by Milagro Granados-Montero, David W. Minter, Rafael F. Castañeda-Ruiz. This brings to 129 the number of free access mycobiota now available on our website: http://www.mycotaxon.com/resources/weblists.html

Central America

Costa Rica

Milagro Granados-Montero, David W. Minter, Rafael F. Castañeda-Ruiz. A check list of asexual fungi from Costa Rica. 41 p.

Abstract—A checklist of recorded asexual fungi from Costa Rica is presented. This study compiles information obtained during 1927—2018 from scientific papers, theses, reviews of hyphomycetous and coelomycetous fungi, dermatophyte clinical studies, clinical samples from phytopathological laboratory services, and specimens collected during the 2012 expedition to Costa Rica conducted in collaboration with the International Society for Fungal Conservation. The taxa included in this checklist represent 682 species and 301 genera. Half have been reported as fungal pathogens, while the remaining are saprobes, endophytes, or associated with insect, human, and other fungal hosts. Forty-six taxa are reported for the first time from Costa Rica.



Tolypocladium dujiaolongae sp. nov.

(Li & al.— Fig. 3, p. 235)